June 1961
VOLUME 83/NUMBER 6

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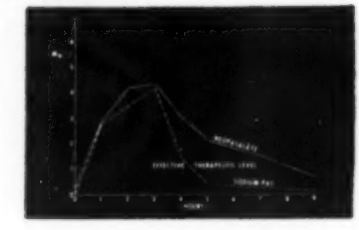

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Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

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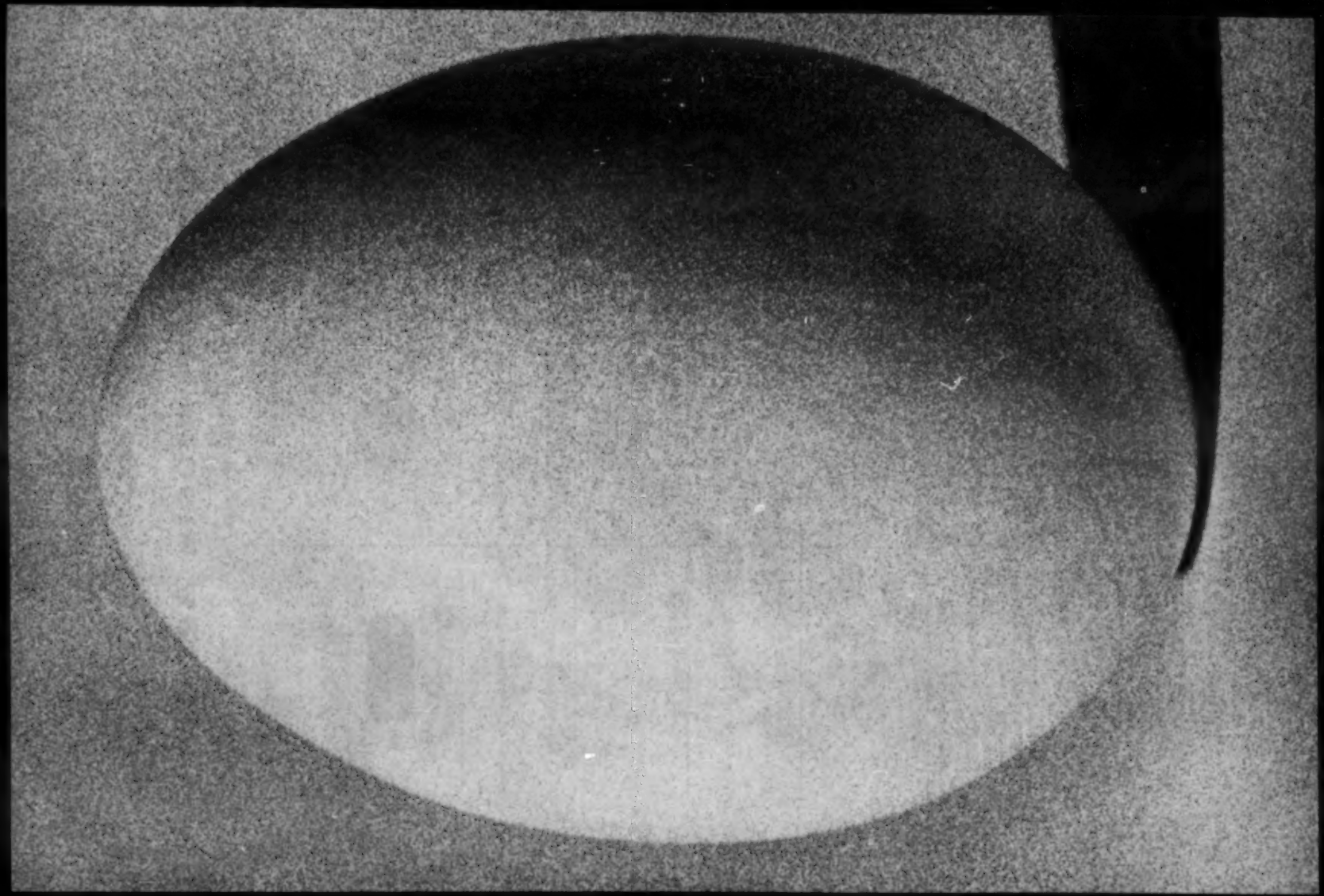

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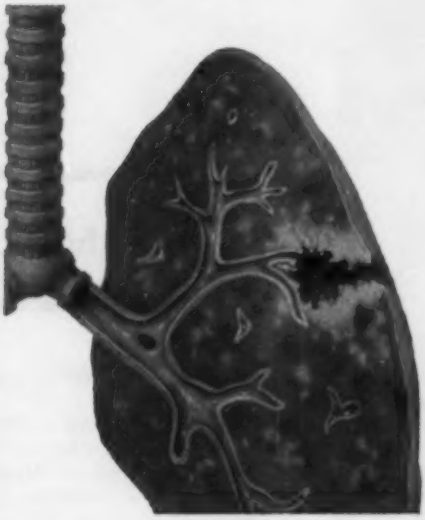

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1. Davey, W. N.: GP 19:107, No. 1, 1959.

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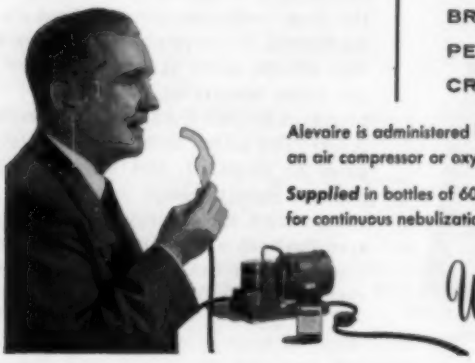

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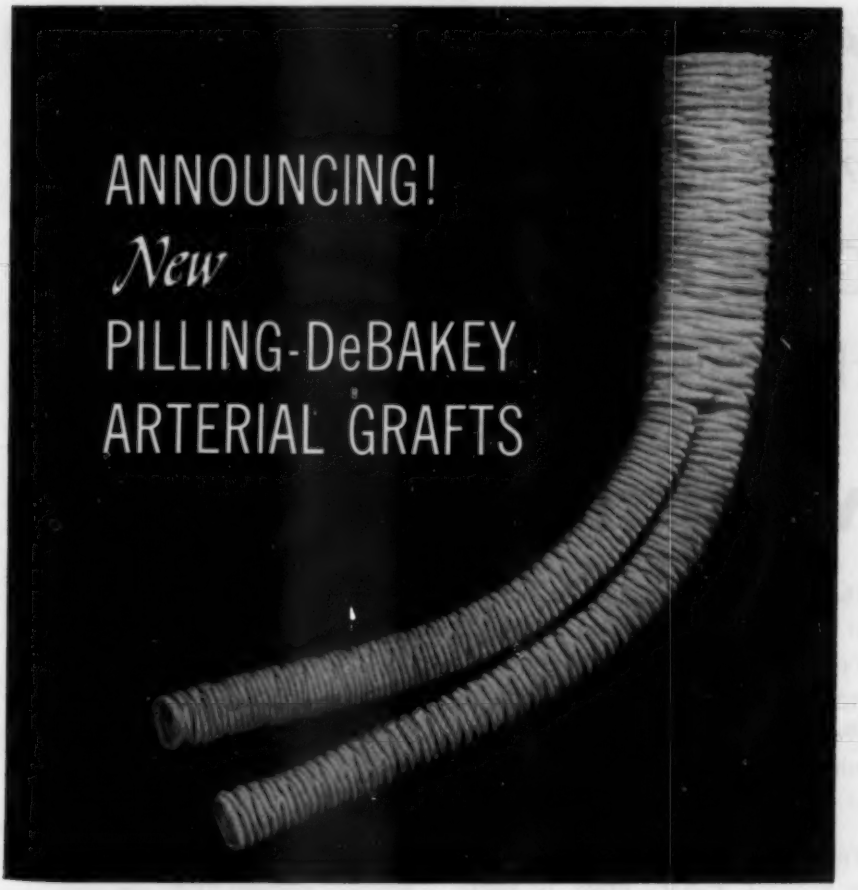

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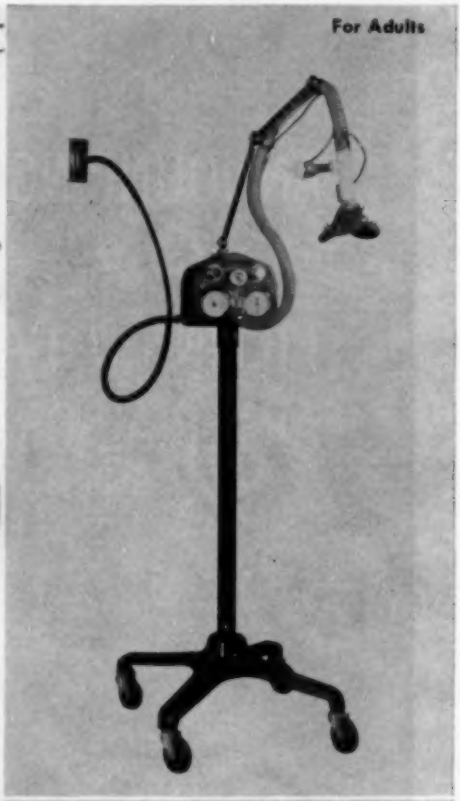

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ERRATUM

In the April, 1961, issue of the REVIEW (volume 83), two corrections should be noted for the "Third Aspen Conference on Emphysema." In the summary of the article, "Air Pollution and Chronic Respiratory Disease," by Stanley N. Rokaw, two references are made to "mean capillary oxygen pressure" on page 572. In both instances, this should have read "*carbon dioxide tension*." Refer specifically to the first paragraph and to point 3 in the third paragraph of this article.

THE AMERICAN REVIEW
OF RESPIRATORY DISEASES

Clinical and Laboratory Studies of Tuberculosis and Respiratory Diseases

VOLUME 83 *June* 1961 NUMBER 6

EVALUATION OF TECHNIQUE AND RESULTS FOR OBTAINING SPUTUM FOR LUNG CARCINOMA SCREENING[1, 2]

A Study by Blind Technique

LINDA LEILOP, MARTA GARRET, AND HAROLD A. LYONS

(Received for publication December 30, 1960)

INTRODUCTION

There is no unanimity of opinion as yet regarding the relative value of the methods used for the detection of lung cancer and application of such methods to an apparently healthy population except that, with any method, once symptoms are present, the diagnosis is usually late. Both roentgenographic screening and cytologic detection are methods currently employed. Thus, the problem is to find which of these methods is the most valuable in revealing lung cancer in any early stage. This report is based upon data accumulated from a survey to assess the relative merits of symptoms, roentgenographic examinations of the chest, and cytologic studies of sputum for screening population groups for the early diagnosis of lung cancer. The practicality of the method, as applied to screening a large population, is also a major consideration.

Patients who have suggestive symptoms are usually found to have advanced malignancy and, if not generalized metastasis, at least regional involvement. Weeks to several months elaspse from onset to development of sufficient symptoms to cause the patient to seek medical advice. Again, the usual procedures of diagnosis for lung carcinoma make for further delay. A considerable number of cases of cancer become unsuitable for treatment because of the delays. The roentgenographic discovery of cancer in an asymptomatic stage has been shown to give the best prognosis after treatment (1). In Sweden an extensive chest roentgenographic study was carried out from 1949 to 1951 on a population of 423,000; 151 cases of lung carcinoma were diagnosed. Eighty-five patients were treated; some survived longer than six years. Those who were untreated died within one or two years. This study demonstrated that if a lung cancer is diagnosed and treated early there will be an improved survival rate.

Opinions vary regarding roentgenographic examination of the chest. On the one hand is the belief that metastases are already present when a suspicious roentgenographic shadow is seen. At the other extreme are those who are convinced that the majority of carcinomas, if found reasonably early by roentgenography, are still localized and, therefore, still suitable for surgical treatment.

The reason for such diverse opinions is the lack of information about the natural history of lung cancer; hence, studies of a large number of patients are required to substantiate the percentage of each form to be established on roentgenography. Cytologic examination of sputum, when strict criteria are adhered to, has been shown to be of considerable value in the diagnosis of pulmonary carcinoma. The procedure may reliably reveal lung cancer in the absence of a roentgenographic abnormality; this is the earliest clinical phase of the disease.

In order to evaluate the merits of the cytologic examination as a screening method, a successful and simple technique is required to obtain sputum from subjects. This is especially true for the majority of healthy persons who

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[2] This study was supported by a grant-in-aid from the American Cancer Society.

usually cannot produce sputum voluntarily, even after vigorous coughing. The method also must be practicable for application to a large number of persons. Fulfilling these requirements, a method using a warmed aerosolized solution of 10 per cent saline and 15 per cent propylene glycol was suggested by Bickerman and co-workers (2). The present study was designed to compare the efficiency of their technique for the production of sputum satisfactory for cytologic examination when different solutions were employed. For this comparison, the saline-glycol mixture, tap water, and normal saline were used separately for aerosolization. These agents were also compared with simple postural drainage.

Materials and Methods

The technique for obtaining satisfactory specimens of sputum for cytologic examination is based on the following observations:

(*a*) Hypertonic solutions in contact with the bronchial mucosa stimulate the secretion of sputum.

(*b*) A warm solution reduces irritation produced by hypertonicity.

(*c*) The solution is best administered as an aerosol mist, the particles of which should be 1 to 10 microns in size. This ensures a mist which on inhalation reaches the bronchioles.

(*d*) The addition of propylene glycol forms a stable and evenly distributed mist to the bronchial mucosa.

The nebulizer designed by Bickerman and associates[3] was used in this study. It best fulfills the specifications listed above. The apparatus consists of a glass jar into which the solution to be aerosolized is placed. A screw top covers the jar. Running through this cover is a heater and thermostatic mechanism enclosed in a metal cylinder which dips into the solution and maintains it at a constant temperature (108° to 110°F.). An outlet for the aerosolized solution passes through this unit. The heater is supplied by an electrical supply. The nebulizer intake connection is attached to a positive pressure pump or a tank of compressed gas (air or oxygen). After the solution becomes heated (within approximately ten minutes), the pump is started. A visible aerosol mist is soon emitted from the nebulizer. A pressure of 5 to 10 ml. of water is usually adequate to obtain a thick mist.

In the present study, the subject sat in a chair and the nebulizer was placed on a table at face level. The mouth was kept about one inch from the outlet tube, and breathing was done with an open mouth. Deep breaths were made every few minutes. The sputum produced from this procedure was then coughed directly into a sterile jar containing 70 per cent alcohol. The mist was inhaled for at least twenty minutes.

Different solutions were used in the nebulizer to compare the effectiveness of each. For this purpose, a solution of glycol and saline, tap water, and normal saline were used. Postural drainage alone was also used to determine whether this method had any value or was superior to the aerosol technique. This procedure was carried out by having the patient hang over a bed with his head and thorax dependent and at an angle of 45° from the horizontal. This position was maintained as long as the patient could tolerate it. A maximal period of twenty minutes was usually attained.

All sputum specimens were sent to the laboratory within a few hours after collection. Whenever early delivery to the laboratory was not possible, the specimens were refrigerated until the delivery.

A blind technique was used for this study. All of the specimens were submitted to the laboratory by number, without name, and without clinical data, in order to eliminate any possible bias in the cytologic interpretation. Some of the specimens were obtained from patients who had had previous cytologic examinations and interpretations. No information was provided to the cytologist about the method or technique employed to obtain the specimen, and the cytologist was completely uninformed of the status of the patient from whom the specimen came.

Five hundred specimens were obtained from 205 patients by the methods described above. All of the subjects were either patients with various pulmonary diseases or apparently healthy persons. For the majority of patients, more than one method was used, and the collections were made on the same day. Whenever postural drainage was used it was usually the first procedure done. Specimens always were obtained a few hours after breakfast, but prior to lunch, in order to minimize contamination with food particles from the mouth. The general attitude and cooperation of all subjects were reasonably good, and no complaints were registered.

A routine Papanicolaou technique was used for the preparation and staining of smears. From each sputum specimen four slides were prepared. No centrifugation of sputum was necessary because the quality of the specimens was comparable to self-produced sputum. The original Papanicolaou classification was used for reporting cytologic findings. In addition to Classes I through V, a "deferred" classification was used in cases in which marked cellular degeneration or unusual features of atypical cells precluded a definite cytologic evaluation.

[3] Manufactured by the O.E.M. Corporation, East Norwalk, Connecticut.

Results

With the different techniques, 500 specimens were obtained from 205 subjects, as shown in table 1. Ninety-six specimens came from apparently normal subjects, 349 from patients with various respiratory diseases, and 55 from patients with suspected respiratory tract malignancies (table 2). All suspicious cases which were later proved to be nonmalignant were finally diagnosed and treated for other pulmonary disease. The over-all results indicate that satisfactory specimens for cytologic examination can be obtained with all of these various techniques.

The presence of alveolar phagocytes containing anthracotic pigment in the smear was considered a criterion for "satisfactory specimens," as this was an indication that the material came from the lower respiratory tract. The best results were obtained with the solution of glycol and saline used as aerosol inhalation. It was found that 88.3 per cent satisfactory specimens were obtained with this agent, whereas 66.6 per cent satisfactory specimens were obtained with self-produced sputum. With other agents, a definitely poorer yield of specimens satisfactory for cytologic examination as compared with the glycol and saline solution method was obtained (table 3). During this study it was noticed that several patients were unable to produce sputum spontaneously, but every patient produced sputum in sufficient quantity for cytologic examination when the glycol and saline aerosol was used. It was subsequently shown that 22 specimens (from 9 patients) of the 55 specimens collected (from 21 patients) with suspected diagnoses of neoplasm of the respiratory tract were reported positive for neoplasm by cytology. These data are shown in table 2. Every positive diagnosis of cancer by cytologic examination was later confirmed by other means: lung biopsy, lung resection, carinal biopsy, scalene node biopsy, or autopsy. The relative efficiency of the various methods on diagnosis of malignancy is shown in table 4. No false positive results (Class IV or V) were obtained with sputum produced by propylene glycol and salt solution aerosol, saline aerosol, or spontaneous sputum. However, the sputum specimens which were obtained during or shortly after postural drainage, even those specimens obtained by other agents, gave false positive results. The cells simulating malignant cells possibly had their origin from inflamed mucosa of the upper respiratory tract. Thus, postural drainage as the sole method, or postural drainage used before any sputum collection, should be avoided. Using the glycol and saline aerosol method, the diagnosis of malignancy was

TABLE 1

Different Techniques for Obtaining Sputum from Patients

	Method Used for Collection						
Sputum	Solution*	Normal Saline	Solution* after Postural Drainage	Postural Drainage	Sputum	Tap Water	Total
Number of specimens	240	98	48	39	54	21	500
Number of subjects	147	84	22	30	42	19	205

* Glycol and saline aerosol

TABLE 2

Specimens from Patients with Various Pulmonary Diseases and Normal Subjects

	Method Used for Collection						
Type of Patients	Solution*	Normal Saline	Solution* after Postural Drainage	Postural Drainage	Sputum	Tap Water	Total
Apparently normal	35	13	13	10	19	6	96
Various pulmonary diseases	174	76	30	24	31	14	349
Suspected lung cancer	31	9	5	5	4	1	55

* Glycol and saline aerosol

TABLE 3

NUMBER AND PERCENTAGE OF SATISFACTORY SPECIMENS FOR CYTOLOGIC EVALUATION
Method Used for Collection

	Solution*	Normal Saline	Solution* after Postural Drainage	Postural Drainage	Sputum	Tap Water	Total
Number of specimens	240	98	48	39	54	21	500
Number of satisfactory specimens	212	78	41	28	36	15	410
Number of unsatisfactory specimens	28	20	7	11	18	6	90
Percentage of satisfactory specimens	88.3	71.7	85.4	71.8	66.6	71.4	82

* Glycol and saline aerosol

made on 6 of the 9 specimens collected on the first use of the method. Two more positive diagnoses were obtained by a second collection for examination. In these 2 instances, the first specimens had been reported as "classification deferred," which required another specimen for diagnosis. Diagnosis was not obtained in one other patient until a third specimen was examined. The use of normal saline aerosol produced only one of five positive reports with the first specimen collection. Postural drainage and use of the glycol and saline aerosol after postural drainage also gave excellent diagnostic results on the first specimens, but because of the occurrence of false positive reports, as mentioned above, this method was discontinued.

DISCUSSION

All of the agents described above can be used for the heated aerosol technique to obtain sputum specimens. However, artifically induced sputum using propylene glycol and hypertonic salt solution aerosol as the agent appears to be superior for the quantity and quality of the specimen submitted for microscopic cytologic evaluation. This agent seems to be more reliable for yielding the greatest number of specimens containing malignant cells. Among the sputum specimens which were self-produced under observation and collected and sent for cytologic studies were those from 2 patients with proved respiratory tract malignancies which repeatedly failed to reveal malignant cells. This finding was in contrast with the fact that both of these patients were promptly diagnosed by specimens obtained via the glycol and saline method.

False positive specimens were reported, but all of these were associated with postural drainage. This finding prompted early discontinuance of this method of collection. It also should be pointed out that, for the patient to be able to cooperate, it is necessary that he have an effective deep cough so as to produce a sufficient amount of material for cytologic study.

No adverse effect occurred from the employment of this technique for collecting sputum.

TABLE 4

COMPARISON OF THE TECHNIQUES FOR OBTAINING FALSE POSITIVE RESULTS
Method Used for Collection

		Solution*		Normal Saline		Solution* after Postural Drainage		Postural Drainage		Sputum		Tap Water	
		Cases	Specimens	Cases	Specimens	Cases	Specimens	Cases	Specimens	Cases	Specimens	Cases	Specimens
Proved	IV	4	4	1	1	—	—	—	—	—	—	1	1
	V	7	10	—	—	2	3	1	1	1	1	—	—
Not proved	IV	—	—	—	—	—	—	1	1	—	—	—	—
	V	—	—	—	—	1	1	—	—	—	—	—	—

* Glycol and saline aerosol

Slight irritation of the mouth and throat was occasionally noted. A few patients were troubled by a cough which lasted no longer than an hour. Blood-tinged sputum remained unchanged, and no hemoptysis occurred after the aerosol inhalation.

Summary

A study and evaluation of the heated aerosol technique for the collection of sputum for the cytologic diagnosis of lung cancer was made, comparing the use of several different solutions with postural drainage alone. A blind technique was used throughout the study.

Postural drainage proved unsatisfactory because of the occurrence of false postive specimens. It was found that, of saline, tap water, and a mixture of propylene glycol and hypertonic saline, the last-named mixture was superior in producing sputum which was adequate and satisfactory for cytologic examination. Of the specimens obtained, 88.3 per cent were satisfactory with the use of this agent, compared to 66.6 per cent obtained with the other agents. No false positive results were obtained, and malignancy was diagnosed in every one of the 9 proved cases of malignancy.

Because the method is simple, yields a high percentage of satisfactory specimens, and is shown to be reliable for diagnosis, it should be given consideration for screening large population groups for lung cancer.

Sumario

Justipreciación de la Técnica y de los Resultados de la Obtención de Esputo para el Despistaje del Carcinoma Pulmonar. Estudio con la Técnica de Eliot

Se hizo un estudio y justipreciación de la técnica de aerosol calentado para la colecta de esputo para el diagnóstico citológico del cáncer pulmonar, comparando el empleo de distintas técnicas con el drenaje postural solo. En todo el estudio se utilizó la técnica de Eliot.

El drenaje postural resultó satisfactorio debido a la existencia de ejemplares seudopositivos. Se observó, usando solución salina, agua del grifo y una mezcla de propileno-glicol y solución salina hipertónica, que la última era superior en lo relativo a producir esputo de índole adecuada y satisfactorio para examen citológico. Con el uso de esta preparación, se obtuvieron 88.3 por ciento de ejemplares satisfactorios, comparado con 66.6 por ciento rendido por los otros agentes. No se observaron seudopositivas y se diagnosticó cáncer en cada uno de los nueve casos comprobados de esta dolencia.

Por ser el método sencillo, rendir un alto porcentaje de ejemplares satisfactorios y haberse demostrado que es fidedigno para diagnóstico, debe tomarse en cuenta al triar grandes grupos de población en busca de cáncer pulmonar.

Resume

Evaluation d'une technique et ses résultats pour obtenir l'expectoration dans le dépistage du carcinome du poumon. Etude d'une technique "aveugle"

Les auteurs effectuèrent une étude et une évaluation de la technique par aérosol chauffé pour récolter les expectorations en vue du diagnostic cytologique du cancer du poumon, comparant l'emploi de plusieurs solutions différentes au drainage de posture seul. Une technique "aveugle" fut employée tout au long de l'étude.

Le drainage postural ne s'avéra pas satisfaisant en raison de la présence d'échantillons faussement positifs. On trouva que, en utilisant les solutions salines, l'eau du robinet, et un mélange de glycol propylène et de solution hypertonique, ce dernier mélange était meilleur pour la production d'une expectoration qui soit adéquate et satisfaisante pour l'examen cytologique. On obtint 88,3 pour cent d'échantillons satisfaisants avec l'emploi de cet agent, comparé à 66,6 pour cent avec les autres agents. On n'obtint aucun résultat faussement positif et on diagnostiqua la malignité dans chacun des neuf cas qui furent prouvés malins.

A cause de sa simplicité, parce qu'elle donne un fort pourcentage d'échantillons satisfaisants et se montre sure pour le diagnostic, cette méthode devrait être considérée pour le dépistage du cancer du poumon dans les larges groupes de population.

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TUBERCULOSIS CONTACTS IN SEOUL, KOREA[1]

An Analysis of 3,002 Household Contacts, 1959

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Introduction

Tuberculosis mortality in South Korea is probably more than 300—and may be as high as 400—per 100,000 population. The few statistics available are inadequate to give an accurate figure. The tuberculin reaction is positive at ten years of age in 60 per cent of the population, at twenty years of age in 80 per cent and, at older than thirty, in 90 per cent. A mass survey of more than 20,000 persons of all ages reported by the Ministry of Health and Social Affairs revealed 3.4 per cent to have active pulmonary tuberculosis. In this clinic 1.7 per cent of 1,150 United States Operation Mission employees or prospective employees were found to have active disease. The rate is probably from 2 to 4 per cent, so there are probably more than 600,000 cases of active disease in South Korea, with its population of 23,000,000 people. As the total number of beds available for the treatment of tuberculosis is 4,000, the establishment of chest clinics and of a system of home care is obviously the only practical method of attack.

Clinics in Seoul

In January, 1954, the Church World Service established a chest clinic in connection with Severance Hospital. This hopsital has been established for seventy-six years and is the oldest hospital in Korea in which modern medicine is practiced. To house the clinic, a building with only three walls standing was reconstructed with material from Korea Civil Assistance Command and cash grants from the United Nations Reconstruction Agency ($21,000) and Church World Service ($6,000).

There were only 7 patients during the first month. With the use of modern chemotherapy, however, the response to treatment was so satisfactory that within seven months the clinic was caring for more than fifty patients a day. At this time another clinic was set up at the Red Cross Hospital in one room of a burnt-out building that in the beginning had no roof, windows, or doors. Funds for the reconstruction of the building were supplied by the American-Korean Foundation ($3,500). By the end of the first year, 1,019 new cases of active pulmonary tuberculosis had been discovered. Other clinics were begun at the City Dongboo Hospital in 1955, Soon Wha Hospital in 1956, and in Yung Dong Po Hospital in 1957. There was a total of 1,626 new cases in 1955 and approximately 2,000 new cases a year for the next four years, making a total of 10,668 active cases of tuberculosis, nearly all of which were newly discovered.

Home Visiting and Relief

From the beginning, the homes of the patients were visited by graduate nurses. By the end of 1959, 20,000 visits had been made to 6,000 homes. The nurses were also responsible for a relief program through which ten tons of food were issued each year to the most needy of the charity patients. In 1959 this service was improved and expanded by the addition of two social workers who gave out 45 tons of food during the year in addition to fuel and clothing.

Area Served

In 1954 the population of Seoul was about 600,000, but by 1959 the number had increased to 1,800,000. There are probably 50,000 cases of active pulmonary tuberculosis in the city alone. Two-thirds of the patients treated come from the city but some live at distances up to 100 miles. The municipal government also conducts clinics so that there are at least 6,000 patients with active tuberculosis who are constantly under treatment in Seoul.

[1] Korea Church World Service Tuberculosis Control Project, Seoul, Korea. Each clinic doctor analyzed his or her own cases.

[2] Present address: American Korean Foundation, A.P.O. 301, % Postmaster, San Francisco, California.

Clinic Routine

In the writers' clinics, the patients are seen by appointment weekly or fortnightly by the same physician. A plan of treatment for each new patient is determined at a daily conference. The physician tries to impress on the patient the need to bring all contacts to the central clinic. After instructions from the physician the patient is referred to a nurse who is also a laboratory technician. She explains more fully the value of rest, method of transmission of tuberculous infection, the need for isolation, et cetera. The patient is given one booklet of general instructions, one on nutrition, and a letter pointing out the need to obtain roentgenograms of the chest of all household contacts. Each contact is provided with a card which entitles the holder to a free 70-mm. chest film. The patient then draws a plan of the location of his or her house which may be on any of the many hills in Seoul and may not have a street address number. On her visit the nurse completes the data required, ages of contacts, et cetera, and again urges that they attend the clinic.

Contacts Examined

In previous years the importance of having all household contacts examined was recognized; 1,243 reported for examination in 1957, and 1,863 in 1958. In 1959, the physicians and nurses at each of the five clinics made a determined effort to persuade all within the city or its environs to attend the central clinic. Nurses visited 1,239 homes and, although 3,992 contacts attended the clinic, complete data are available for only 3,526 contacts of 900 index cases. The number examined roentgenographically was 3,002 (85.1 per cent) (tables 1–3; figure 1). The new active cases found numbered 357 (11.9 per cent). Fourteen per cent of these were believed to represent source cases. The source case of a contact was the case of tuberculosis judged by

TABLE 1

Attendance at Hospitals

Hospital	Number of Index Cases	Number of Contacts	Contacts with Roentgenograms		Contacts with Pulmonary Tuberculosis	
			Number	Per Cent	Number	Per Cent
Severance Hospital	444	1,767	1,593	90.2	170	10.7
Red Cross Hospital	191	657	529	80.1	78	14.4
Soon Wha Hospital	26	117	97	82.9	12	12.4
Yung Dong Po City Hospital	149	687	548	79.8	67	12.3
Dongboo City Hospital	90	298	238	79.9	30	12.6
Total	900	3,526	3,002	85.1	357	11.9

TABLE 2

Household Contacts: Attendance by Age and Sex

Age Group	Male			Female			Both Sexes		
	Total Number	Nonattendance	Per Cent Attendance	Total Number	Nonattendance	Per Cent Attendance	Total Number	Nonattendance	Per Cent Attendance
0–4	281	40	85.8	252	39	84.5	533	79	85.2
5–9	265	30	88.7	265	33	87.7	533	63	88.2
10–19	530	53	90.0	436	48	89.0	966	101	89.5
20–29	177	32	81.9	302	31	89.7	479	63	86.6
30–39	132	24	81.8	235	27	88.5	367	51	85.8
40–49	111	27	75.7	194	28	86.0	305	55	82.0
50–59	67	30	55.2	151	35	76.8	218	65	70.2
60–69	63	22	65.1	62	25	59.7	125	47	62.4
Total	1,626	258	84.7	1,900	266	86.0	3,526	524	85.1

TABLE 3

TUBERCULOSIS MORBIDITY RATES OF HOUSEHOLD CONTACTS ACCORDING TO AGE AND SEX

Age	Number of Contacts			Number with Pulmonary Tuberculosis			Per Cent with Pulmonary Tuberculosis		
	M	F	Total	M	F	Total	M	F	Total
0–4	241	213	454	38	24	62	15.8	11.3	13.4
5–9	235	235	470	13	18	31	5.5	7.7	6.6
10–19	477	388	865	44	47	91	9.2	12.1	10.5
20–29	145	271	416	33	29	62	22.7	10.7	14.8
30–39	108	208	316	22	21	43	20.4	10.1	13.6
40–49	84	166	250	16	16	32	19.0	9.6	12.8
50–59	37	116	153	10	10	20	27.0	8.6	13.1
60+	41	37	78	12	4	16	29.3	10.8	20.5
Total	1,368	1,634	3,002	188	169	357	13.7	10.3	11.9

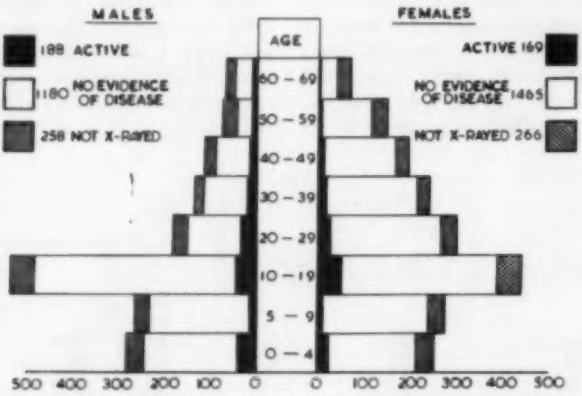

FIG. 1. Pulmonary tuberculosis household contacts of patients attending five clinics in Seoul, 1959. Total contacts: 3,526; number examined roentgenographically, 3,002; age and sex composition.

the physician to be the most probable source of infection for that particular contact after taking into consideration all of the information available about various members of the household and other members of the contact group (1).

In 65 per cent of the diseased contacts the tuberculosis was "minimal"; in 26.3 per cent, moderately advanced; and in only 8.7 per cent was it far advanced. By contrast, the classification of the disease of the index cases was "minimal" in only 11.8 per cent, moderately advanced in 38.2 per cent, and far advanced in 50 per cent (figure 2).

DISCUSSION

By persistent effort it was possible to achieve 85.1 per cent acceptance of contact examination among families of Korean patients, even though many had to travel long distances, sometimes 100 miles, to the central clinic. The attendance of contacts of patients attending the Severance Clinic was 90.2 per cent. Apparently the better the clinic and the longer in operation, the better the response. The percentage of contacts with active tuberculosis (11.9) was three to five times the number of active cases of tuberculosis usually found by mass roentgenographic surveys of the general population. It is therefore a more useful and cheaper method of case finding.

It might be expected that the spread of tuberculosis in families would be affected by the number of individuals per room, the size of the room, and the intimacy of contact. An analysis of records for 1,000 homes showed that 60 per cent of the families lived in one room only, and 6 per cent of these single rooms were only 6 feet

	NO OF CASES	MINIMAL	MOD. ADV.	FAR ADV.
INDEX	900	11.8	38.2	50.0
CONTACT	357	65.0	26.3	8.7

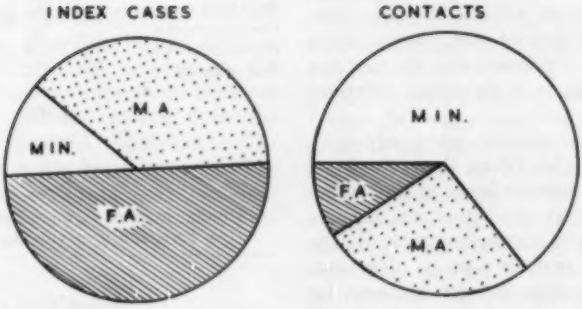

FIG. 2. Pulmonary tuberculosis: five clinics in Seoul, 1959. Classification of index cases and household contacts.

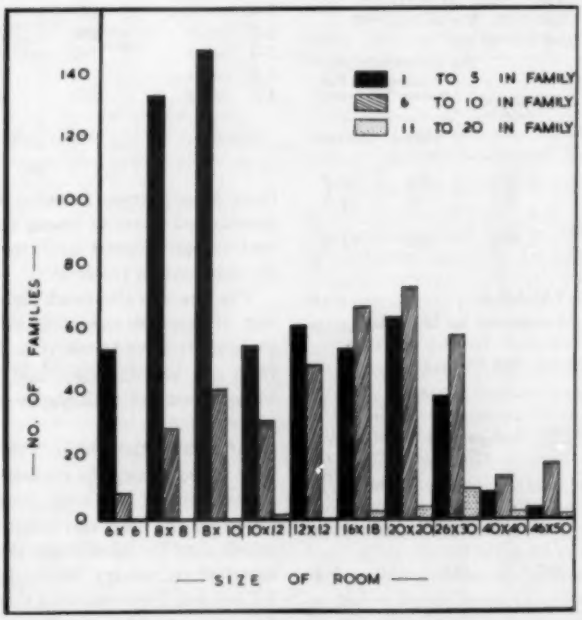

FIG. 3. Area of living space per family.

square. Twenty-six per cent had two rooms, and less than 1 per cent had five rooms. The average living space was 120 square feet for a family of five persons (figure 3). The morbidity rate of 11.9 per cent is more than double that in the poorer areas of several western countries, where the average occupancy per room is 1.3 persons. It has been stated that in some western countries the morbidity rate is not influenced by the standard of housing. In the present survey, however, the morbidity of those living in the same room as the patient was 12.3 per cent, whereas, if the patient lived in a separate room, the rate was 9.0 per cent. This is a significant difference (table 4).

As would be expected, more active pulmonary tuberculosis was found among those in contact with persons with tubercle bacilli in the sputum than among those whose sputum was negative for tubercle bacilli on microscopy (table 5). The rate was 13.5 per cent for contacts of patients with infectious sputum and 5.8 per cent for patients with sputum negative for tubercle bacilli on microscopy. The high rate of infection from grandparents is noted because they frequently take care of young children and represent the group least likely to attend the clinic for examination (table 6).

The rate was also much higher when the duration of exposure exceeded two years. It was 8.7 per cent at seven to twelve months, 12.5 per cent from one to two years, and 14.7 per cent for those in contact with the source case over four years (table 7).

Fifty cases thought to represent source cases were found among the contacts. This constituted 14 per cent of the total number. As might be expected, the more advanced the disease in the source case the more likely were the contacts to manifest pulmonary tuberculosis. The rate was 5.3 per cent for contacts of those with "minimal" disease and 14.5 per cent for those in contact with patients with far advanced disease (table 8).

TABLE 4

PULMONARY TUBERCULOSIS MORBIDITY IN HOUSEHOLD CONTACTS ACCORDING TO ROOM LIVED IN

Room	Number of Contacts	Contacts with Pulmonary Tuberculosis	
		Number	Per Cent
Same room	2,301	294	12.3
Different room	701	63	9.0
Total	3,002	357	11.9

TABLE 5

TUBERCULOSIS MORBIDITY IN HOUSEHOLD CONTACTS ACCORDING TO THE SPUTUM STATUS OF THE SOURCE

Sputum Status	Number of Source Cases	Number of Contacts	Contacts with Pulmonary Tuberculosis	
			Number	Per Cent
Positive on microscopy	713	2,383	321	13.5
Negative on microscopy	187	619	36	5.8
Total	900	3,002	357	11.9

TABLE 6

TUBERCULOSIS MORBIDITY RATES OF HOUSEHOLD CONTACTS ACCORDING TO RELATION OF CONTACT

Relationship of Contact	Number of Contacts Examined	Contacts with Pulmonary Tuberculosis	
		Number	Per Cent
Consort	460	58	12.6
Parents	1,581	191	12.1
Brothers or sisters	693	82	11.8
Grandparents	38	7	18.4
Other relatives	178	15	8.4
Not related	52	4	7.7
Total	3,002	357	11.9

TABLE 7

TUBERCULOSIS MORBIDITY OF HOUSEHOLD CONTACTS ACCORDING TO THE DURATION OF EXPOSURE TO THE SOURCE CASES

Duration	Number of Source Cases	Number of Contacts	Contacts with Pulmonary Tuberculosis	
			Number	Per Cent
1–6 months	74	244	11	4.5
7–12 months	170	569	56	9.8
1–2 years	246	818	102	12.5
2–3 years	163	558	71	12.7
3–4 years	110	323	45	13.9
4+ years	137	490	72	14.7
Total	900	3,002	357	11.9

The source of infection for 86 children who developed primary tuberculosis was the parents in 76 instances, other siblings in 6, grandparents in 6, and was unknown in one instance. Boys less than five years of age showed a higher incidence of infection than girls of the same age. At puberty the morbidity among females was higher than among males of the same age. For males more than twenty years old the tuberculous disease rate was higher than the female rate and was particularly high (28 per cent) for males more than fifty-one years old who were contacts of infectious cases. The old people have the highest rate for nonattendance at the clinic for examination as household contacts. From the standpoint of treatment and prognosis, it was encouraging to find that the disease was in an early state in 65 per cent of contacts with active disease and that in only 8.7 per cent was the tuberculosis far advanced (table 9).

TABLE 8

CONTACTS WITH PULMONARY TUBERCULOSIS EXTENSION OF DISEASE OF SOURCE CASES

Extension of Disease	Number of Source Cases	Number of Contacts	Contacts with Pulmonary Tuberculosis	
			Number	Per Cent
Primary	1	5	0	0
Minimal	75	245	13	5.3
Moderately advanced	354	1,174	111	9.5
Far advanced	470	1,578	233	14.5
Total	900	3,002	357	11.9

TABLE 9

CLASSIFICATION OF PULMONARY TUBERCULOSIS INDEX AND CONTACT CASES

Extension of Disease	Index Cases		Contact Cases	
	Number	Per Cent	Number	Per Cent
Primary tuberculosis	1	0.1	89	24.9
Minimal	105	11.7	143	40.1
Moderately advanced	344	38.2	94	26.3
Far advanced	450	50.0	31	8.7
Total	900	100.0	357	100.0

SUMMARY

An analysis is presented of the household contacts of 900 "index cases" who attended five chest clinics in Seoul in 1959. The total number of contacts was 3,526. Among these contacts, 3,002 (85.1 per cent) were examined roentgenographically, and 357 (11.9 per cent) were found to have active pulmonary tuberculosis. Fourteen per cent of these were believed to represent source cases.

Nonattendance at the clinics was high in those more than fifty and, particularly, in those more than sixty years of age. It is in this latter group that many hidden source cases were found.

Morbidity rates were closely related to the extent of the disease, the bacteriologic status of the source case, the duration of exposure, the contact's relationship to the source case and, specifically, whether the two persons lived in the same or different rooms. In primary tuberculosis, the parents were the main source of the infection. The pulmonary tuberculosis was minimal in 65 per cent of the new cases found on examination of contacts. This represented a most favorable type of disease from the standpoint of what might be accomplished by chemotherapy. The importance of examining household contacts, particularly the older age group, has again been demonstrated.

SUMARIO

Contactos Tuberculosos en Seúl, Corea. Un Análisis de 3,002 Contactos Caseros, 1959

Preséntase un análisis de los contactos caseros de 900 "casos índices" que asistieron a cinco clínicas torácicas de Seúl en 1959. El número total de contactos fué 3,526. De éstos, se examinó radiográficamente a 3,002 (85.1 por ciento), y en 357 (11.9 por ciento) se descubrió tuberculosis pulmonar activa. Un 14 por ciento de éstos eran casos focales.

La inasistencia a las clínicas fué alta en las personas de más de cincuenta años, y en particular en las de más de sesenta. Es en este último grupo que se descubren muchos casos focales ocultos.

Las tasas de morbidad se relacionan íntimamente con la difusión de la enfermedad, el estado bacteriológico del caso focal, la duración de la exposición y la relación del contacto con el caso focal; y también si viven en el mismo cuarto o en aposentos separados. En la tuberculosis primaria, los padres son la causa principal de la infeción.

Los nuevos casos descubiertos fueron en 65 por ciento de enfermedad mínima, permitiendo el tra-

tamiento temprano. Se ha demostrado una vez más la importancia de examinar los contactos caseros, y en particular el grupo de edad avanzada.

Resume

Contacts tuberculeux à Séoul, Chorée. Une Analyse de 3,002 contacts de maisonnée

Les auteurs présentent une analyse des contacts de maisonnée de 900 cas "index" qui fréquentèrent cinq consultations pour les maladies de poitrine à Séoul en 1959. Le nombre total de contacts fut de 3.526. Parmi ces contacts, 3,002 (85,1%) furent examinés à la radio, et chez 357 (11,9%), on trouva une tuberculose pulmonaire active. Quatorze pour cent de ceux-ci étaient des cas "source".

Parmi ceux-ci, l'irrégularité des visites à la consultation fut grande, plus de cinquante, et particulièrement parmi les malades ayant dépassé soixante ans. C'est dans ce dernier groupe qu'on découvrit plusieurs cas sources occultes.

Les taux de morbidité sont en relation étroite avec l'extension de la maladie, l'état bactériologique du cas source, la durée d'exposition, et le rapport de contact avec le cas source; et aussi s'ils vivent dans une même chambre ou à part. Dans la tuberculose primaire, les parents sont la source principale d'infection.

On trouva de nouveaux cas-65% de maladie à minimum, ce qui rend possible un traitement précoce. L'importance de l'examen des contacts de maisonnée, surtout dans le groupe des gens agés, a été démontrée à nouveau.

Acknowledgment

The figures were prepared by Mrs. Yung Sun Kim, a clinic nurse.

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SOME IMPLICATIONS OF THE ADDITION OF TWEEN® 80 TO DILUTIONS OF PPD TUBERCULIN WITH RESPECT TO THE STUDY OF "SPECIFIC" AND "NONSPECIFIC" TUBERCULIN SENSITIVITY[1]

A Preliminary Report

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Introduction

In the latter part of 1958, a new batch of human PPD tuberculin, designated by the producers (the State Serum Institute of Copenhagen, Denmark) as RT 23, was released. It was recommended that this tuberculin be diluted in a phosphate buffer containing 0.005 per 100 ml. Tween® 80. As it was not realized at first that the addition of Tween 80 might be of great importance to dilutions of PPD tuberculin other than RT 23, the phosphate buffer containing Tween was, for reasons of simplicity, made the standard diluent for all comparative tuberculin studies then in progress in the territory. It soon became evident, however, that tuberculins containing Tween 80 were much stronger than anticipated. Accordingly, a number of studies on the effect of the addition of Tween were designed to clarify this situation. Although it may still take considerable time before these studies are completed, it was thought worth while to report some of the preliminary observations at this time.

Materials and Methods

Tuberculin: All of the tuberculins used in this study were recieved in the form of stock solutions containing 50,000 TU per ml. The tuberculin RT 23 was received from the State Serum Institute in Copenhagen; PPD-Seibert was obtained from the U.S. Public Health Service through the courtesy of Dr. Phyllis Q. Edwards.

Dilutions were prepared locally once a week and were kept refrigerated until used. Fresh dilutions were used each working day.

Syringes: Only new syringes were used for comparative tuberculin testing. They were pretested for leakage by the method of Guld and Rud (1), and only those that leaked less than 0.2 ml. after being subjected to an internal pressure of 5.5 kg. per cm.^{2} for six minutes were used. All of the syringes were marked with colored tapes in order to avoid possible exchange, and the bottles containing the corresponding tuberculins were marked in the same way.

Reduction of "volume effect": As it is known that tuberculins which do not contain Tween tend to give smaller reactions if the vial in which the tuberculin is stored is only partly filled (2, 3), only one-half of the completely filled bottle of tuberculin was used. When tuberculins diluted in phosphate buffer with and without Tween were compared, only one-half of those containing Tween were used.

Injection technique: All tuberculin injections were given on the dorsum of the forearms, approximately one-third of the distance from the wrist or one-third from the elbow. When several injections were given at the same time, the site of the injection was randomized to make sure that the reader was unaware of what reaction he was reading.

One-tenth of a milliliter of fluid was used for all tuberculin tests, and the injections were given intradermally as accurately as possible according to the markings of the barrel (4).

Reading of reactions: All reactions were read after seventy-two hours. No attempt was made to classify them as either positive or negative, but the palpable induration was carefully measured with a calibrated ruler, and the mean of the transverse and the longitudinal diameters was recorded.

Unless stated otherwise, all reactions were read by the same reader.

Observations

PPD Tuberculin RT 23

Comparison between 1 TU of RT 23 with Tween and 2.5 TU of RT 23 without Tween:

In a population living at a high altitude in a district where clinical tuberculosis is unknown: In October, 1959, 36 adult males from a remote highland district (altitude approximately 4,800 feet above sea level) where clinical tuberculosis is unknown were tuberculin tested and examined roentgenographically a few days after arrival in Hollandia by air. A number of comparative tests were given, including 1 TU of RT 23 with Tween and 2.5 TU of RT 23 without Tween. The chest roentgenograms did not reveal pulmo-

[1] From the Tuberculosis Control Division, Department of Public Health, Hollandia, Netherlands New Guinea.

CORRELATION TABLE 1

COMPARISON BETWEEN 1 TU OF RT 23 WITH TWEEN AND 2.5 TU OF RT 23 WITHOUT TWEEN In a Population Living at a High Altitude in a District Where Clinical Tuberculosis is Unknown

Size of Reaction to 1 TU of RT 23 with Tween 80	Size of Reaction to 2.5 TU of RT 23 without Tween												
	0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	20–	22+	Tota
0–	–	1	6	5	3	2	–	1	–	–	–	–	18
2–	–	–	1	1	–	–	–	–	–	–	–	–	2
4–	–	–	1	1	1	1	–	–	–	–	–	–	4
6–	–	–	–	–	2	–	1	–	–	–	–	–	3
8–	–	–	–	–	2	–	2	–	–	–	–	–	4
10–	–	–	–	–	–	–	1	1	–	–	–	–	2
12–	–	–	–	–	1	–	1	–	–	–	1	–	3
14+	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	–	1	8	7	9	3	5	2	–	–	1	–	36

nary disease. The results of the tests with RT 23 are presented in correlation table 1. As may be seen, the mean reaction to 2.5 TU of RT 23 without Tween was larger than the mean reaction to 1 TU of RT 23 with Tween.

In a population, living at a low altitude in a district where clinical tuberculosis is unknown: In March, 1960, comparative tuberculin studies were made in the district of Ajamaroe. The altitude of the district is approximately 350 feet above sea level. Although clinical tuberculosis has not yet been found in the district of Ajamaroe,[2] some of the adult males had worked on the coast for short terms and some may have acquired an infection with tubercle bacilli while there. The results of the tests with RT 23 in a sample of the general population (dental age I and above[3]) are given in correlation table 2. In these subjects also there was a larger mean reactivity to 2.5 TU of RT 23 without Tween than to the duplicate test of 1 TU of RT 23 with Tween. As compared with the results in the adult males from the former district, the sensitivity to both tests was greater.

In patients suffering from clinical tuberculosis (bacteriologically confirmed diagnosis only): In September, 1959, 44 patients newly admitted to the tuberculosis wards of the Central Hospital at Hollandia were given duplicate tests employing 2.5 TU of RT 23 without Tween and 1 TU of RT 23 with Tween. The results are given in correlation table 3. As may be seen, in contrast to the observations described for the subjects living in districts where clinical tuberculosis is unknown, the reactions to both tests were approximately the same size in the patients with demonstrable disease. There was some indication that the reactions to 1 TU of RT 23 with Tween might have been slightly larger than those to 2.5 TU of RT 23 without Tween.

In a coastal district where clinical tuberculosis is common[4]*:* In November, 1959, a general population group in the district of Kaimana (dental

[2] It should be noted that a district medical officer has been working in the district which has a population of 17,000 people (since 1955) and that intensive patroling, with physical examinations of the total population, has been carried out each year since 1955. Yaws has been eradicated. The attendance of the population at these yearly surveys, although somewhat decreasing, is still approximately 85 per cent. No case of tuberculosis has ever been found.

[3] As the peoples of Netherlands New Guinea very often do not know their exact age, and as it is difficult to assess the actual age of the people examined, it is common practice within the Department of Public Health to determine age in terms of dental age. Thus, the last tooth to appear is recorded for everybody examined (5).

[4] On Reformation Day (October 31, 1959) some 2,000 of the indigenous population of the district assembled in the township of Kaimana to celebrate the occasion. As it is unlikely that the sick people were as eager to participate in the festivities as the healthy ones, the sample cannot be regarded as representative for the district as a whole. From the 2,000 people tested, 659 showed very strong reactions to 1 TU of RT 23 with Tween. All 659 persons were examined by fluoroscopy, and 20 cases of pulmonary densities suggestive of active pulmonary tuberculosis were found.

CORRELATION TABLE 2

COMPARISON BETWEEN 1 TU OF RT 23 WITH TWEEN AND 2.5 TU OF RT 23 WITHOUT TWEEN
In a Population Living at a Low Altitude in a District Where Clinical Tuberculosis is Unknown*

Size of Reaction to 1 TU of RT 23 with Tween 80 \ Size of Reaction to 2.5 TU of RT 23 without Tween	0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	20–	22+	Total
0–	45	12	4	3	6	6	2	3	1	–	–	–	82
2–	3	7	3	–	1	–	–	1	–	–	–	–	15
4–	–	3	7	4	3	3	3	–	–	–	–	–	23
6–	3	–	2	–	3	2	6	1	–	–	–	–	17
8–	–	–	–	–	2	1	5	3	1	–	–	–	12
10–	–	–	–	–	–	2	4	4	1	–	–	–	11
12–	–	–	–	–	–	1	4	2	3	1	–	–	11
14–	–	–	–	–	–	1	3	6	5	2	1	–	18
16–	–	–	–	–	–	–	–	2	3	2	–	–	7
18–	–	–	–	–	–	–	–	1	1	1	1	–	4
20+	–	–	–	–	–	–	–	–	–	–	–	–	—
Total	51	22	16	7	15	16	27	23	15	6	2	–	200

* *See* footnote 2 in text.

CORRELATION TABLE 3

COMPARISON BETWEEN 1 TU OF RT 23 WITH TWEEN AND 2.5 TU OF RT 23 WITHOUT TWEEN
In Patients with Clinical Tuberculosis (Bacteriologically Confirmed Diagnosis Only)

Size of Reaction to 1 TU of RT 23 with Tween 80 \ Size of Reaction to 2.5 TU of RT 23 without Tween	0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	20–	22–	24–	26–	28+	Total
0–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–	2
16–	–	–	–	–	–	–	–	1	4	2	2	–	–	–	–	9
18–	–	–	–	–	–	–	1	–	1	4	4	–	–	–	–	10
20–	–	–	–	–	–	–	–	–	5	3	5	–	–	–	–	13
22–	–	–	–	–	–	–	–	–	2	1	1	1	–	–	–	5
24–	–	–	–	–	–	–	–	–	–	–	2	–	1	1	–	4
26–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
28–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1
30+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	–	–	–	–	–	–	1	3	12	10	14	2	1	1	–	44

CORRELATION TABLE 4

COMPARISON BETWEEN 1 TU OF RT 23 WITH TWEEN AND 2.5 TU OF RT 23 WITHOUT TWEEN
In a Population Living in a District Where Clinical Tuberculosis is Common

Size of Reaction to 1 TU of RT 23 with Tween (S) \ Size of Reaction to 2.5 TU of RT 23 without Tween	0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	20–	22–	24–	26–	28–	30+	Total
0–	35	4	7	5	3	5	6	5	1	–	–	–	–	–	–	–	71
2–	8	6	11	4	–	1	–	–	–	–	–	–	–	–	–	–	30
4–	2	2	4	2	2	3	4	1	–	–	–	–	–	–	–	–	20
6–	2	–	1	1	1	2	2	–	–	–	–	–	–	–	–	–	9
8–	–	1	–	1	2	7	4	4	1	–	–	–	–	–	–	–	20
10–	–	1	1	–	–	2	8	4	1	–	–	–	–	–	–	–	17
12–	–	–	–	–	–	1	9	12	5	–	–	–	–	–	–	–	27
14–	–	–	–	–	–	–	1	18	7	1	1	–	–	–	–	–	28
16–	–	–	–	–	–	–	–	11	25	10	2	–	–	–	–	–	48
18–	–	–	–	–	–	–	–	11	10	20	5	1	–	–	–	–	47
20–	–	–	–	–	–	–	–	–	10	15	8	2	–	–	–	–	35
22–	–	–	–	–	–	–	–	1	1	2	4	4	–	2	–	–	14
24–	–	–	–	–	–	–	–	–	–	1	–	3	1	1	–	–	6
26–	–	–	–	–	–	–	–	–	–	1	–	1	1	–	–	–	3
28–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	1
30+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	47	14	24	13	8	21	34	67	61	50	21	11	2	3	–	–	376

age I and higher), where clinical tuberculosis is common, were given duplicate tests of 1 TU of RT 23 with Tween and 2.5 TU of RT 23 without Tween. The results are given in correlation table 4.

As may be seen in the table, persons with small and medium reactions (up to 12 to 15 mm. in size) to 1 TU of RT 23 with Tween had larger reactions to 2.5 TU of the same tuberculin without Tween. But persons with strong reactions to 1 TU of RT 23 with Tween (16 mm. and more) had reactions of approximately the same size to 2.5 TU without Tween.

Comparison between 20 TU of RT 23 with Tween and 50 TU of RT 23 without Tween:

A number of comparative tuberculin studies had made it likely that the people from the district of Ajamaroe all had sensitivity of a non-specific nature to tuberculin, e.g., not acquired through infections with human tubercle bacilli but through infections of another organism, presumably a Mycobacterium. Accordingly, 20 TU of RT 23 with Tween and 50 TU of RT 23 without Tween were given to a sample of the population as duplicate tests, along with the standard dose of 1 TU of RT 23 with Tween. No unfavorable reactions were seen and the results are given in correlation table 5. As may be seen from the data in the table, the reaction to 50 TU of RT 23 without Tween was stronger than the reaction to 20 TU of RT 23 with Tween.

Human PPD-Seibert (PPD-S)

Comparison between 5 TU of PPD-S without Tween and 2 TU of PPD-S with Tween:

In a population living at a low altitude in a district where clinical tuberculosis is unknown: A sample of the general population of Ajamaroe (dental age I and higher), mentioned earlier, were given duplicate tests with 5 TU of PPD-S without Tween and 2 TU of PPD-S with Tween. The results are given in correlation table 6.

CORRELATION TABLE 5

COMPARISON BETWEEN 20 TU OF RT 23 WITH TWEEN AND 50 TU OF RT 23 WITHOUT TWEEN
In a Population Living at a Low Altitude in a District Where Clinical Tuberculosis is Unknown*

Size of Reaction to 20 TU of RT 23 with Tween 80	Size of Reaction to 50 TU of RT 23 without Tween																
	0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	20–	22–	24–	26–	28–	30+	Total
0–	9	1	2	–	1	3	–	2	–	–	1	–	–	–	–	–	19
2–	–	–	1	–	1	1	–	1	1	–	–	–	–	–	–	–	5
4–	–	–	–	–	1	1	1	–	1	–	–	–	–	–	–	–	4
6–	–	–	–	–	–	1	5	–	2	–	–	–	–	–	–	–	8
8–	–	–	–	–	–	–	2	–	1	–	–	–	–	–	–	–	3
10–	–	–	–	–	–	–	2	2	4	–	–	–	–	–	–	–	8
12–	–	–	–	–	–	–	1	2	4	1	–	–	–	–	–	–	8
14–	–	–	–	–	–	–	–	1	6	3	1	–	–	–	–	–	11
16–	–	–	–	–	–	–	–	–	7	5	9	–	1	–	–	–	22
18–	–	–	–	–	–	–	–	–	4	3	4	2	1	–	–	–	14
20–	–	–	–	–	–	–	–	–	–	2	4	5	1	–	–	–	12
22–	–	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–	2
24–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
26–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	–	2
28–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
30+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	9	1	3	–	3	6	11	8	30	14	21	7	4	–	1	–	118

* *See* footnote 2 in text.

CORRELATION TABLE 6

COMPARISON BETWEEN 2 TU OF PPD-S WITH TWEEN AND 5 TU OF PPD-S WITHOUT TWEEN
In a Population Living at a Low Altitude in a District Where Clinical Tuberculosis is Unknown

Size of Reaction to 2 TU of PPD-S with Tween 80	Size of Reaction to 5 TU of RT 23 without Tween												
	0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	20–	22+	Total
0–	74	9	8	8	3	3	2	3	2	–	–	–	112
2–	3	4	2	1	–	–	1	–	–	–	–	–	11
4–	3	2	2	1	1	–	–	1	–	–	–	–	10
6–	–	–	–	–	–	–	–	–	–	–	–	–	–
8–	2	–	–	–	–	–	–	–	–	–	–	–	2
10–	–	–	–	–	–	1	–	–	–	–	–	–	1
12–	–	–	–	–	–	–	3	–	–	1	–	–	4
14–	–	–	–	–	–	–	–	–	1	–	–	–	1
16–	–	–	–	–	–	–	–	–	2	–	–	–	2
18–	–	–	–	–	–	–	–	–	–	–	–	–	–
20+	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	82	15	12	10	4	4	6	4	5	1	–	–	143

Results of a somewhat similar nature to the comparison between 1 TU of RT 23 with Tween and 2.5 TU of RT 23 without Tween (*see* correlation table 2) were seen. The reaction to 5 TU of PPD-S without Tween was somewhat stronger than to the duplicate test of 2 TU of PPD-S with Tween.

In patients suffering from clinical tuberculosis (only patients with tubercle bacilli in sputum by microscopy or culture are included): In July, 1960, 27 patients who were admitted to the tuberculosis wards in the Central Hospital and from whom tubercle bacilli had been recovered were tested with 5 TU of PPD-S without Tween and 2 TU of PPD-S with Tween. The results are given in correlation table 7.

The reaction to both tests was much the same. The size of the reactions to the 2-TU tests tended to be somewhat larger than those to 5 TU.

In a coastal district where clinical tuberculosis is common: In May, 1959, the population of the island of Noemfoor was examined by mass roentgenography. In 1.7 per cent of the population (dental age M1 and higher), roentgenographic densities suggestive of active tuberculosis were found. A sample of the population (dental age I and higher) received duplicate tests with 5 TU of PPD-S without Tween and 2 TU of PPD-S with Tween. The reactions were read by a different reader and the results are presented in correlation table 8.

Again, the large reactions tended to be of the same size with both tests, whereas persons who were smaller or medium reactors to 2 TU of PPD-S with Tween had larger reactions to 5 TU of PPD-S without Tween. The pattern was very similar to that found in Kaimana for 1 TU of RT 23 with Tween and 2.5 TU of RT 23 without Tween.

Other PPD Tuberculins

At present, further observations are being made with a number of other tuberculins, including some not prepared from *Mycobacterium tuberculosis* var. *hominis*. Although these studies are still in progress, the results thus far tend to

CORRELATION TABLE 7

COMPARISON BETWEEN 2 TU OF PPD-S WITH TWEEN AND 5 TU OF PPD-S WITHOUT TWEEN
In Patients with Clinical Tuberculosis (Bacteriologically Confirmed Diagnosis Only)

Size of Reaction to 2 TU of PPD-S with Tween	Size of Reaction to 5 TU of PPD-S without Tween																
	0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	20–	22–	24–	26–	28–	30+	Total
0–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
6–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
10–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
12–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
14–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–	1
16–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	1
18–	–	–	–	–	–	–	–	–	2	2	1	1	–	–	–	–	6
20–	–	–	–	–	–	–	–	–	–	1	3	3	–	–	–	–	7
22–	–	–	–	–	–	–	–	–	–	1	2	1	–	2	–	–	6
24–	–	–	–	–	–	–	–	–	–	–	2	2	–	–	–	–	4
26–	–	–	–	–	–	–	–	–	–	1	–	1	–	–	–	–	2
28+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	–	–	–	–	–	–	–	–	3	6	8	8	–	2	–	–	27

CORRELATION TABLE 8

COMPARISON BETWEEN 2 TU OF PPD-S WITH TWEEN AND 5 TU OF PPD-S WITHOUT TWEEN
In a District Where Clinical Tuberculosis is Common

Size of Reaction to 2 TU of PPD-S with Tween	Size of Reaction to 5 TU of PPD-S without Tween																	
	0–	2–	4–	6–	8–	10–	12–	14–	16–	18–	20–	22–	24–	26–	28–	30–	32+	Total
0–	20	8	4	1	–	1	3	–	1	–	–	–	–	–	–	–	–	38
2–	5	9	4	3	1	1	2	–	–	–	–	–	–	–	–	–	–	25
4–	–	1	1	–	–	1	–	–	–	–	–	–	–	–	–	–	–	3
6–	–	–	–	–	–	1	1	2	–	–	–	–	–	–	–	–	–	4
8–	–	–	–	–	1	1	–	2	–	1	–	–	–	–	–	–	–	5
10–	–	–	–	–	–	–	1	1	–	1	–	–	–	–	–	–	–	3
12–	–	–	–	1(?)	–	–	1	1	2	2	2	–	–	–	–	–	–	9
14–	–	–	–	–	–	–	–	2	3	3	1	–	1	–	–	–	–	10
16–	–	–	–	–	–	–	–	2	–	2	1	1	–	–	–	–	–	6
18–	–	–	–	–	–	–	–	–	2	11	4	3	2	–	–	–	–	22
20–	–	–	–	–	–	–	–	–	–	2	4	2	1	–	–	–	–	9
22–	–	–	–	–	–	–	–	–	–	–	4	4	2	–	–	–	1	11
24–	–	–	–	–	–	–	–	–	–	–	1	–	2	1	–	–	–	4
26–	–	–	–	–	–	–	–	–	–	–	1	–	2	–	–	–	–	3
28–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
30–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1
32+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	25	18	9	5	2	5	8	10	8	22	18	10	10	2	–	–	1	153

be very much the same as those described above for RT 23 and PPD-S.

DISCUSSION

From the observations presented in this paper it is evident that, in Netherlands New Guinea, persons infected with true tubercle bacilli (pulmonary lesions with demonstrable tubercle bacilli in sputum) have reactions of approximately the same size to a low-dose tuberculin test containing Tween 80 as they have to 2.5 times as much of this same tuberculin in a diluent not containing Tween (*see* correlation tables 3 and 7).

This observation is in agreement with recent reports that PPD tuberculin is normally adsorbed to a certain extent to the wall of its container, but that this adsorption can be prevented through the addition of a small amount of Tween 80 to the diluent (6).

In districts where clinical tuberculosis is unknown, however, and where it is presumed that the population has acquired tuberculin sensitivity through a cause(s) other than an infection with true tubercle bacilli, the reaction to a low-dose tuberculin test containing Tween is markedly less than to 2.5 times as much of the same tuberculin not including Tween (*see* correlation tables 1, 2, and 6).

Finally, when the tuberculin sensitivity of general population groups in districts where clinical tuberculosis is common is studied, a pattern is found which resembles a simple addition of the pattern found in persons with true tuberculous disease plus that found in districts where clinical tuberculosis is unknown. Smaller reactors to the tuberculin containing Tween have larger reactions to 2.5 times as much of the same tuberculin not containing Tween. However, individuals who react to the tuberculin containing Tween with reactions approximately equal in size to those commonly found in cases of tuber-

culosis have reactions of approximately the same size to 2.5 times as much of the same tuberculin not containing Tween (*see* correlation tables 4 and 8).

All of these observations suggest that each PPD tuberculin is composed of at least two fractions or groups of fractions, as has been suggested in a paper on the standardization of RT 23 (7), in which it is stated:

> "There is reason to believe that PPD is composed of at least two fractions of different adsorbability, one that has a low adsorbability and is found even in ordinary dilutions of PPD, and one that is highly adsorbable and is therefore found mainly or exclusively in stabilized dilutions of PPD. A possible explanation of the present findings is that these factors differ also in regard to specificity; thus it might be assumed that the highly adsorbable fraction is the more specific one, in the sense that it does not elicit reactions in persons whose allergy is of 'nonspecific' origin."

As the phenomenon described in detail for RT 23 and PPD-S in the present report can also be found with another human PPD—and something very similar has been seen with avian PPD prepared from different strains, Battey tuberculin, and bovine PPD—it seems justified to assume that the majority of PPD tuberculins, regardless of the Mycobacterium from which they are prepared, are composed of two fractions or groups of fractions.

There are certain implications of this finding, one of which has to do with tuberculin sensitivity acquired through previous contact with true tubercle bacilli.

In the search for sensitivity caused by infections with true tubercle bacilli, the addition of Tween 80 to the diluent of human PPD seems to be advantageous because it permits us to use a much lower concentration of PPD tuberculin, and nonspecific reactions, which are so frequent in many tropical countries, are less likely to occur. In addition, some of the unpublished observations of the present writer indicate that Tween in itself diminishes the frequency of these nonspecific reactions, particularly in a low-dose tuberculin test.

For these two reasons it can be anticipated that in tropical regions, where both nonspecific and specific tuberculin sensitivity is prevalent, the distribution of reactions to a low-dose tuberculin test containing Tween will be more bimodal than the distribution of reactions to 2.5 times as much of the same tuberculin not containing Tween. The "natural break" between "positives" and "negatives" will therefore be more clear if Tween is used in the diluent of human PPD.

The present studies in the subjects with proved tuberculous disease indicate that some 60 per cent of the fraction which is regarded to be the more specific of the two is normally adsorbed to the wall of the container. This is in agreement with earlier reports (6). Why should those who work in areas of the world where "cross-reactions" or "nonspecific" reactions to tuberculin are common not use this phenomenon of selective adsorption in the improvement of the specificity of the tuberculin test. If a vial containing a solution of an appropriate concentration of human PPD without Tween were emptied, and the adsorbed and presumably more specific fraction were redissolved, there would not only be a rather high yield of specific fraction (60 per cent as compared with 40 per cent in the original solution not containing Tween), but the less desirable and less adsorbable fractions of the PPD would have been disposed of.

Thus far, only a very few observations on this matter have been made by the writer, but the results obtained lend support to the abovementioned theoretic consideration that it is possible to improve the specificity of the tuberculin test in this way.

Another implication has to do with tuberculin sensitivity not acquired through infections with true tubercle bacilli (the reaction commonly called "nonspecific" tuberculin sensitivity). In the study of this type of sensitivity, the addition of Tween 80 to the diluent of the tuberculin may be a disadvantage. This type of sensitivity is much less intense than the so-called "specific" tuberculin sensitivity, which is acquired through infection with true tubercle bacilli. Therefore, a low-dose tuberculin test that elicits well-defined and rather strong reactions in persons who have been infected with true tubercle bacilli shows either no reaction or only a rather weak reaction in persons who have acquired tuberculin sensitivity of a "nonspecific" nature.

A high-dose tuberculin test, containing twenty times as much tuberculin as the low dose, is, therefore, commonly used for the demonstration of "nonspecific" tuberculin sensitivity in

those persons who have either no reaction or only a relatively weak reaction to the low dose.

As could be demonstrated in the present material (*see* correlation tables 1, 2, 5, and 6) "nonspecific" tuberculin sensitivity seems to be associated to a greater degree with the nonadsorbable fractions in PPD tuberculin. Therefore, it seems that there is little reason to add Tween to the diluent of a high-dose tuberculin test. Furthermore, as there is reason to believe that Tween in itself reduces the size of some tuberculin reactions in persons who have not been infected with true tubercle bacilli, but who have acquired their sensitivity to tuberculin through another cause, there seems even less point in adding Tween 80 to the diluent of our high-dose tuberculin test.

If it be presumed that the different tuberculins, RT 23, RT 22, and PPD-S, all contain relatively the same proportions of adsorbable and nonadsorbable material, then 20 × 2.5 TU, or 50 TU of RT 23 not containing Tween, would be more or less comparable to 100 TU of RT 22 (which was the tuberculin in use in the UNICEF-assisted BCG vaccination campaigns until RT 23 was released), or 100 TU of PPD-S. A twenty fold concentration of the standard low-dose test of 1 TU of RT 23 with Tween, e.g., 20 TU of RT 23 with Tween, would not be comparable to the earlier standard of 100 TU of RT 22 without Tween.

In data thus far unpublished, there is some indication that RT 23 contains relatively more (or possibly more potent) nonadsorbable tuberculin than the previous batch of RT 22 or PPD-S. Therefore, 40 TU of RT 23 without Tween may be approximately the concentration which is comparable to the previous high dose of 100 TU of RT 22.

Summary

Duplicate tests of human PPD in a diluent containing 0.05 per cent of Tween 80 and 2.5 times as much of the same tuberculin diluted in phosphate buffer not containing Tween were given to: a general population in a district where clinical tuberculosis is unknown, a general population in a district where clinical tuberculosis is common, and persons with bacteriologically proved cases of tuberculosis.

The patients with proved tuberculosis and persons who had reactions of the same size as are normally found in clinical tuberculosis had reactions of approximately the same size to both tests. Persons who had reactions of a size such as are commonly found in districts where clinical tuberculosis is unknown, however, had much smaller reactions to the tuberculin containing Tween than to 2.5 times as much of the same tuberculin not containing Tween.

The study, therefore, seems to confirm earlier reports that PPD tuberculin is composed of at least two fractions, one of high and one of low adsorbability. The addition of Tween to the diluent of human PPD enhances the tuberculin reaction in persons infected with true tubercle bacilli, but it also decreases the size of some "nonspecific" reactions. For these two reasons, the distribution of a low-dose tuberculin test containing Tween is more bimodal than the distribution of 2.5 times as much of the same tuberculin not containing Tween, e.g., 2 TU of PPD-S with Tween and 5 TU of PPD-S without Tween. In the search for persons infected with true tubercle bacilli, the addition of Tween 80 to the diluent of the low-dose tuberculin test is, therefore, advantageous. The distribution of reactions according to size will show a better separation between persons who are presumably infected and persons presumably not infected with true tubercle bacilli.

In the study of nonspecific tuberculin sensitivity, however, it is argued that the addition of Tween 80 is a disadvantage. In such reactors it seems better to continue to use a phosphate buffer not containing Tween as the diluent of human PPD. Formerly, a twenty fold concentration of the low-dose tuberculin test was used as a high-dose tuberculin test. Now, after the addition of Tween 80 to the diluent of the low dose of PPD, and the consequent reduction in the amount of tuberculin actually dissolved, a fifty fold concentration of this dose in a diluent not containing Tween seems appropriate for the high-dose tuberculin reaction.

Sumario

Algunas Connotaciones de la Adición de Tween® 80 a Diluciones de Tuberculina DPP con Respecto al Estudio de la Sensibilidad "Específica" y "Anespecífica" a la Tuberculina

Se hicieron pruebas en duplicado del DPP humano en un diluente que contenía 0.05 por ciento de Tween 80 y 2.5 veces más de la misma tuberculina diluída en amortiguador fosfático que no contenía Tween, en: la población general de un

distrito en el que se desconoce la tuberculosis clínica, la población general de un distrito en el que es frecuente la tuberculosis clínica y en personas con casos de tuberculosis comprobada bacteriológicamente.

Los enfermos con tuberculosis comprobada y las personas que tuvieron reacciones del mismo tamaño que las que se encuentran normalmente en la tuberculosis clínica mostraron reacciones aproximadamente del mismo tamaño en ambas pruebas. Sin embargo, las personas que tuvieron reacciones aproximadamente del mismo tamaño que las que se observan comúnmente en los distritos en que se desconoce la tuberculosis clínica mostraron reacciones mucho menores a la tuberculina que contanía Tween que a una cantidad 2.5 veces mayor de la misma tuberculina que no contenía Tween.

El estudio confirma, por lo tanto, comunicaciones anteriores, en el sentido de que la tuberculina DPP está compuesta a lo menos de dos fracciones, una de alta y una de baja adsorbibilidad. La adición de Tween al diluente del DPP humano acrecienta la reacción a la tuberculina en personas infectadas con verdaderos bacilos tuberculosos, pero también decrece el tamaño de algunas reacciones "anespecíficas." Por estas dos razones, la distribución de una tuberculina a baja dosis que contiene Tween es más bimodal que la distribución de una dosis 2.5 veces mayor de la misma tuberculina sin Tween (p. ej., 2 UT de DPP-S con Tween y 5 UT de DPP-S sin Tween). En la busca de personas infectadas con verdaderos bacilos tuberculosos, resulta, por consiguiente ventajosa la adición de Tween 80 al diluente de tuberculina reactiva a baja dosis. La distribución de las reacciones conforme al tamaño revelará una separación mejor entre las personas que presuntamente están infectadas y las personas que presuntamente no están infectadas con verdaderos bacilos tuberculosos.

No obstante, se arguye que, en el estudio de la sensibilidad anespecífica a la tuberculina, la adición de Tween 80 constituye una desventaja. En esos reactores, parece mejor continuar usando como diluente del DPP humano un amortiguador fosfático que no contenga Tween. Anteriormente, se usaba para la prueba de la tuberculina a alta dosis una concentración veinte veces mayor de la tuberculina de baja dosis. Hoy día, después de agregar el Tween 80 al diluente de la dosis baja del DPP, y la consiguiente reducción en la cantidad de tuberculina realmente disuelta, una concentración cincuenta veces mayor de dicha dosis en un diluente que no contenga Tween parece apropiada para la reacción de la tuberculina a alta dosis.

Resume

Quelques implications de l'addition de Tween 80 à des dilutions de tuberculine PPD, par rapport a l'étude de la sensibilité "spécifique" et "non spécifique" a la tuberculine

Avec une tuberculine humaine PPD, dans une dilution contenant 0,05% de Tween 80 et 2,5 fois autant de même tuberculine diluée dans une solution-tampon phosphatée, ne contenant pas de Tween, on fit des examens en double, chez une population generale, dans une region ou la tuberculose clinique etait inconnue, dans une population générale dans une région où la tuberculose est commune, et chez des personnes qui sont des cas prouvés bactériologiquement.

Les malades avec une tuberculose prouvée et les personnes qui eurent des réactions de la même taille que celle que l'on trouve dans la tuberculose clinique, eurent des reactions ayant a peu près la même taille dans les deux examens. Les personnes qui eurent des réactions de la taille qui est trouvée communément dans les regions ou la tuberculose clinique est inconnue, eurent, cependant, des réactions beaucoup plus petites à la tuberculine contenant du Tween, qu'a 2,5 fois plus de la même tuberculine sans Tween.

L'étude, par conséquent, confirme les rapports précédents à savoir que la tuberculine PPD est composée d'au moins deux fractions, une de forte et une de faible adsorbabilité. L'addition de Tween à la dilution de PPD humaine exagère la réaction a la tuberculine chez les personnes infectées avec de vrais bacilles tuberculeux, mais ceci diminue aussi la taille de quelques réactions "non specifiques." Pour des deux raisons, la distribution d'une réaction a une petite dose de tuberculine contenant du Tween est plus ambivalente que la distribution de 2 fois et demi plus de la même tuberculine sans Tween (c. à.d., 2 TU de PPD-S avec Tween and 5 TU de PPD-S sans Tween). Dans la recherche de personnes infectées avec de vrais bacilles tuberculeux, l'addition de Tween 80 à la dilution d'une faible dose de la tuberculine de réaction est par consequent avantageuse. La distribution des réactions selon la taille montrera une meilleure séparation entre les personnes qui sont supposées infectées et celles qui ne sont pas supposées infectées avec de vrais bacilles tuberculeux.

Dans l'étude de la sensibilité non spécifique à la tuberculine, cependant, on discute le fait que l'addition de Tween 80 est un désavantage. Dans de tels produits de réaction, il semble qu'il vaut mieux continuer à utiliser une solution tamponnée avec du phosphate ne contenant pas de Tween, tel que la dilution de PPD humaine. Auparavant, une

concentration au vingtieme de tuberculine de réaction à faible dose était utilisée comme réaction a une forte dose de tuberculine. Maintenant, après addition de Tween 80 à la dilution de la faible dose de PPD, et la réduction secondaire dans la quantité de tuberculine pratiquement dissoute, une concentration au cinquantième de cette dose dans une dilution sans Tween semble convenir à la réaction a la forte dose de tuberculine.

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EPIDEMIOLOGIC STUDIES OF OBSTRUCTIVE VENTILATORY DISEASE OF THE LUNG[1]

II. A Follow-back Approach for Studying Mortality from Obstructive Ventilatory Diseases of the Lung: Results of a Pilot Study

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INTRODUCTION

Since 1950, a rapid rise has been recorded in death rates for a group of chronic respiratory diseases. These diseases include asthma, bronchiectasis, chronic bronchitis, pulmonary fibrosis,[3] and pulmonary emphysema. The name "obstructive ventilatory disease of the lungs" has been suggested for this group of disorders (1). The data on the occurrence in California are shown in table 1. However, this increase has also occurred in other states and in the United States as a whole. Within this group, the most striking increase has been in deaths reportedly due to pulmonary emphysema, and it is this increase which is of special relevance because deaths formerly attributed to this cause were relatively few.

While death certificates show an increased number of deaths in which these conditions are the *underlying* cause of death, they are also frequently cited on death certificates as "contributory" causes of death. The contributory cause of death in this context is defined as any of these respiratory diseases mentioned on the death certificate but not coded as the underlying cause of death. The California State Department of Public Health's Multiple Cause Coding Study (3), based on 1955 data obtained from the National Office of Vital Statistics, disclosed that for every case in which one of these diseases was mentioned as an underlying cause of death, there were 1.7 cases in which only one was mentioned as a contributory cause (table 2). Information about the trend in specified contributory causes of death is not available.

Faced with a sharp rise in a particular disease category, it becomes necessary to determine whether or not the rise is real and, if so, what factors are contributing to the rise. In investigating the significance of this reported increase, it was necessary to start with the diagnostic criteria physicians use to certify these diseases on death certificates. It was also necessary to examine the relationship of these diseases to environmental factors, such as occupation, cigarette smoking, and air pollution exposures, as determined by residence histories. Before undertaking a full-scale study, it seemed advisable to conduct a pilot study to determine the most efficient and economical investigative techniques for compiling the needed information.

Hence, a methodologic study was undertaken to compare the efficiency of mail versus personal interview techniques in studying the diagnostic criteria used by physicians when certifying that chronic obstructive respiratory diseases are either underlying or contributory causes of death. The comparison of mail with personal interview methods is important because the latter is more costly.

PLAN OF INVESTIGATION

The first phase of the study was concerned with the underlying causes of death; the second, with the contributory causes. The relative efficiency of mail versus personal interviews in obtaining environmental data from next of kin was also evaluated.

All interviewing was done in the Los Angeles area because of the population concentration there. The study was conducted by the State Department of Public Health with the approval of the Los Angeles County Medical Association, the California Osteopathic Association, and all city and county health officers in the area.

General method: A panel of chest disease specialists was consulted to determine the type of questions to ask certifying physicians in order to study the criteria used in making a given diagnosis.

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[3] Cases of fatal pulmonary fibrosis are usually found in death registration data under the category "other chronic interstitial pneumonia"; 525 is the number of this entity in the International Statistical Classification of Diseases, Injuries, and Causes of Death (2).

TABLE 1

DEATHS DUE TO SELECTED RESPIRATORY DISEASES IN CALIFORNIA

(Rates are per 100,000 Population)

Respiratory Disease	1950	1951	1952	1953	1954	1955	1956	1957	1958
Total selected respiratory diseases									
Total number	910	1,014	1,241	1,331	1,433	1,498	1,736	1,978	2,279
Rate	8.6	9.2	10.3	10.9	11.4	11.5	12.8	14.0	15.4
Asthma (241)*									
Total number	336	369	479	491	442	422	460	467	398
Rate	3.2	3.3	4.1	4.0	3.5	3.2	3.4	3.3	2.7
Emphysema (527.1, 502.0)									
Total number	161	196	257	341	392	459	592	803	926
Rate	1.5	1.7	2.2	2.8	3.1	3.5	4.4	5.7	6.3
Bronchitis (500, 501, 502.1, 525, 526)									
Total number	413	449	478	499	509	617	684	708	955
Rate	3.9	4.1	4.1	4.1	4.8	4.7	5.0	5.0	6.5

* Numbers in parentheses represent International Statistical Classification Code (sixth revision).

Source: U.S. Bureau of the Census, United States Census of Population: 1950, vol. II: General Characteristics of the Population, California; and State of California, Department of Finance, Population Estimates, 1951–1958.

TABLE 2

CHRONIC RESPIRATORY DISEASES IN CALIFORNIA, UNDERLYING VERSUS CONTRIBUTORY CAUSES, 1955*

Cause of Death	ISC†	Underlying Cause	Contributory Cause	Ratio: Contributory to Underlying
Total		726	1,236	1.7
Asthma	241	223	260	1.2
Bronchitis, unqualified	501	19	35	1.8
Bronchitis with emphysema	502.0	19	19	1.0
Other chronic bronchitis	502.1	23	75	3.3
Other chronic interstitial pneumonia	525	126	193	1.5
Bronchiectasis	526	96	157	1.6
Emphysema without bronchitis	527.1	220	497	2.3

* Based on multiple cause coding of 50 per cent of the California deaths.

† International Statistical Classification.

The questionnaire schedules were then developed, with the assistance of consultants skilled in survey methods. The same questionnaire was used for both phases of physician interviewing, except for adding response categories of "none" or "not applicable" to some questions in the second phase. The questionnaire is shown in figure 1. Sampling was carried out according to the following plan:

From the death certificates filed for September and October, 1958, for the County of Los Angeles, a sample of forty cases was selected from 110 certificates on which one of the chronic obstructive respiratory diseases was listed as the underlying cause of death. The criteria of selection are detailed below. The certificates selected were randomly divided into two groups within each disease category. One group was to receive a mail questionnaire; the other group was to be personally interviewed, using the same questionnaire.

A second sample of forty-eight death certificates was selected for the period May 15 to June 30, 1959, to explore the problem of chronic obstructive respiratory disease as a contributory cause of death. These death certificates were also divided into a mail questionnaire group and a personal interview group. The following sampling criteria were used:

1. Decedents less than thirty-five years old were excluded because it was believed that these cases did not represent the chronic respiratory diseases included in the study.

2. Death certificates signed by a coroner were excluded because it was believed that the in-

formation provided by the coroner's office would be no different in a mail questionnaire than in a personal interview.

3. Certificates signed by pathologists from the Veterans Administration hospitals were also excluded since in 95 per cent of these cases the

FIG. 1.
Physician Questionnaire

California Respiratory Disease Mortality Study—State Department of Public Health

Name of Deceased: _____ Age: _____ Death Certificate Code No.: _____

Date of Death: _____

After you have reviewed the folder for the deceased, will you please answer all the questions below in as much detail as possible? Please write the word "none" or "not applicable" if question does not apply to this case.

1. For how long was the deceased under your care? _____ years, or, _____ months.

2. What signs and symptoms did you consider pertinent in making your diagnosis of chronic respiratory disease? (e.g., cough, sputum, hemoptysis, wheezing, increased A.P. diameter, dyspnea, decreased mobility of diaphragm, rales, peripheral edema, fever, cyanosis, etc.)

3. What facts in the deceased's history did you consider pertinent in making your diagnosis of chronic respiratory disease? (e.g., smoking, occupation, previous illness, etc.)

4. What were the radiological findings?

5. What laboratory studies did you consider pertinent in making your diagnosis? (e.g. blood picture, biopsy reports, bacteriological studies, cytologic reports, etc.)

6. What therapeutic tests were pertinent in making your diagnosis? (e.g., antispasmodics, antibiotics, other)

7. What other findings did you consider pertinent in making your diagnosis? (bronchoscopy, pulmonary function tests, autopsy reports, E.K.G., etc.)

8. Which of the above data (questions 2 through 7) did you consider the more important ones in making the diagnosis of chronic respiratory disease in this patient's case?

(*Fig. 1 continued*)

9. The following is a copy of the cause of death section of the death certificate signed by you:

	30. CAUSE OF DEATH		
	PART I. DEATH WAS CAUSED BY: IMMEDIATE CAUSE (A)		
CAUSE			APPROXIMATE INTERVAL BETWEEN ONSET AND DEATH
OF	Conditions, if any which gave rise to the above cause (A) stating the underlying cause last. DUE TO (B)		
	DUE TO (C)		
DEATH	PART II. OTHER SIGNIFICANT CONDITIONS CONTRIBUTING TO DEATH BUT NOT RELATED TO THE TERMINAL DISEASE CONDITION GIVEN IN PART I (A)		

The State Department has coded _____ as the underlying cause of death. Do you agree?

☐ Yes ☐ No

IF NO:

What would you have preferred as the underlying cause of death?

_____

10. Remarks: _____

cause-of-death section is completed on the basis of the gross autopsy findings (4), and therefore the basis for the diagnosis is known.

4. A 16 per cent subsample for deaths in which bronchopneumonia was listed as the underlying cause of death was included because it was believed that acute bronchopneumonia might have been the terminal illness listed as the cause of death in cases of chronic respiratory diseases.

The same criteria were used for the contributory cause groups except that deaths from bronchopneumonia were excluded unless some chronic respiratory disease was also mentioned on the certificate. Cases in which the physicians had been certifying physicians for the underlying cause group were excluded from the contributory cause group as it was believed that they would not provide any additional information concerning the criteria which they used.

The distribution of cases selected for study of underlying causes and contributory causes is shown in tables 3 and 4, respectively.

Interviewing methods: Information concerning diagnostic criteria was collected from certifying physicians using the questionnaire shown in figure 1. Clearance to interview the next of kin was obtained from the certifying physician by requesting him to indicate if the next of kin should not be interviewed. Personal and environmental history data were obtained from the person designated as the informant on the death certificate, using the schedule shown in figure 2. Except in two cases, this was the next of kin. Death certificates were randomly divided into mail and personal interview groups, except for cases in which the informant was listed as out-of-state. These, of necessity, were assigned to the mail questionnaire group. Next-of-kin interviews were carried out only for the underlying cause group, since this group was sufficient to answer the questions on comparative efficiency of mail and personal interviews.

To evaluate the questionnaires more effectively, a randomly preselected subsample of the mail questionnaire groups (physicians and next of kin) was personally interviewed. Consistency of answers was determined, and any difficulties or ambiguities in the questionnaires were discussed with the respondents.

Evaluating uniformity of criteria used: Completed physician questionnaires for the first 37 cases in which a chronic obstructive respiratory disease was the underlying cause of death were sub-

TABLE 3

CASES SELECTED FOR STUDY WITH CHRONIC RESPIRATORY DISEASE AS UNDERLYING CAUSE OF DEATH
September–October, 1958, Los Angeles County

Disease Categories of all Certificates	Total Number of Certificates Available	Cases Excluded because of Age of Patients	Cases Excluded because of Coroner's Certificate	Cases Excluded by Veterans Administration Pathologist	Cases Excluded for Contributory Causes	Cases in Sample
Asthma (241)*	12	1	2	0	0	9
Bronchopneumonia (491)	55	7	2	1	3	7†
Chronic bronchitis (502.2)	0	0	0	0	0	0
Bronchiectasis (526)	9	0	0	5	0	4
Other chronic interstitial pneumonia (525)	11	5	0	1	0	5
Pulmonary emphysema	23	0	0	8	0	15
Totals	110	13	4	15	3	40

* Numbers represent International Statistical Classification Code (sixth revision).
† Seven cases were randomly selected from the 42 remaining in this category.

TABLE 4

CASES SELECTED FOR STUDY WITH CHRONIC RESPIRATORY DISEASES AS CONTRIBUTORY CAUSES OF DEATH
May–June 1959, Los Angeles County

Disease Categories	Total Number of Certificates Available	Cases Excluded because of Age of Patient (less than 35)	Cases Excluded because of Coroner's Certificate	Cases Excluded because of Autopsy Performed by Veterans Administration Hospital	Cases Excluded because Physician Participated in Underlying Cause Group	Cases in Sample
Asthma	16	0	1	0	1	14
Chronic bronchitis	4	0	0	0	1	3
Bronchiectasis	7	0	0	1	1	5
Other chronic interstitial pneumonia	5	0	0	0	0	5
Pulmonary emphysema	35	0	0	11	3	21
Totals	67	0	1	12	6	48

mitted to a panel of chest consultants who were asked to fill out the schedule in figure 3. They did not know the certifying physician's cause-of-death entry, nor did they know which questionnaires were from the mail group and which were from the personal interview group.

RESULTS

Physicians who specified one of the chronic obstructive respiratory diseases as the underlying cause of death were questioned first. Nine of twenty questionnaires mailed were returned within two weeks; another was returned just prior to the follow-up letter which was sent one month after the initial mailing. Only one physician failed to respond to the follow-up letter. He was contacted by telephone, and a personal interview was arranged. A total of nine questions on six questionnaires was unanswered. These were completed by telephone calls to the physicians involved. Seventeen of the originally scheduled twenty personal interviews were completed in two weeks; the remainder was completed within the following month. Five preselected physicians were personally interviewed following the return of their completed mail questionnaires. This was done partly to determine whether the delay in completion was due to a basic difficulty of the questionnaire. Interviews with the one physician who did not respond and with the preselected physicians indicated that the questionnaire was not the reason

for delay. As a result, a more sustained follow-up procedure was planned.

In the second phase, physicians who had listed a chronic obstructive respiratory disease as a contributory cause were interviewed. Two questionnaires from the mail group were returned blank, one physician stating that the death in question had nothing to do with chronic respiratory disease. This physician was interviewed personally. In the second case, the physician thought the questionnaire was for the next of kin. This questionnaire was returned to him to fill out. Seventy-five per cent of the questionnaires were returned within three weeks. A second questionnaire was mailed to the six physicians who had failed to respond, and four were returned within one week. One of the four respondents suggested contacting another physician who was more familiar with the respiratory condition of the deceased. The two nonrespondent physicians were contacted by telephone, and a third questionnaire was mailed to them. Because a statement had been added to the questionnaire asking the physician to state "none" or "not applicable" if a particular question did not apply, the number of blanks was reduced to two each on two questionnaires. Again, these were completed by telephone. At the close of the

FIG. 2. Schedule used in interviewing next of kin.

CALIFORNIA MORTALITY SURVEY

STATE DEPARTMENT OF PUBLIC HEALTH

2151 Berkeley Way, Berkeley, California

Interviewer _____

Length of Interview _____ Hrs. _____ Min.

Date of Interview _____

File Number _____ Physician's Name _____

1. Identification of Informant:	2. Identification of Deceased:
Name	Name
Street Address	Street Address
City, State	City, State
Telephone Number	Sex / Race / Age
Relationship to Deceased	Date of Death
3. Person Interviewed:	4. Reason for Interview with Substitute Respondent:
☐ Designated Informant ☐ Substitute Respondent	
Name	
Street Address	
City, State	
Telephone Number	
Relationship	

(Fig. 2 continued)

PART I
Residence History of the Deceased
List only places with residences of one year or more.

A. What was (_____)'s usual place of residence at time of death?

Street or R.F.D. Address

City

County, State

B. Was the residence inside the limits of this city?
☐ Yes ☐ No ☐ Don't know

C. Was the residence on a farm?
☐ Yes ☐ No ☐ Don't know

D. When did the (_____) move to this address?
_____ Month _____ Year

E. Did (_____) live in this city all of his life?
☐ Yes ☐ No

F. Did (_____) move to this place for health or medical reasons?
☐ Yes ☐ No

If yes, what was the reason?

G. When did he move to this city?
_____ Month _____ Year

		Years? From	To	Inside City Limits? Yes	No	DK	On Farm? Yes	No	DK	Move for Health or Medical Reasons? Yes	No
RESIDENCE BEFORE LAST?	City County, State			☐	☐	☐	☐	☐	☐	☐	☐ If yes, reason?
BEFORE THE ABOVE?	City County, State			☐	☐	☐	☐	☐	☐	☐	☐ If yes, reason?
BEFORE THE ABOVE?	City County, State			☐	☐	☐	☐	☐	☐	☐	☐ If yes, reason?
BEFORE THE ABOVE?	City County, State			☐	☐	☐	☐	☐	☐	☐	☐ If yes, reason?
BEFORE THE ABOVE?	City County, State			☐	☐	☐	☐	☐	☐	☐	☐ If yes, reason?

Where was (_____) born? City _____ County, State or Foreign Country _____

How long did he live there? _____ Years

(Fig. 2 continued)

PART II
Job History of the Deceased
List all jobs held in order requested.

A. When was the last time that (_____) worked?

☐ Never worked

_____ Year

_____ Month, if in 1957 or 1958

B. What kind of work did he do?

C. In what industry or business did he work?

D. Employer's Name

City

County and State

E. How long did he work at this job for this employer?

From _____ To _____

F. Did he give up this job for health or medical reason?

☐ Yes ☐ No If yes, what reason?

G. List all jobs held for one or more years most recent one first.

Years From	Years To	Kind of Work?	Industry or Business	City and State	Leave for Health or Medical Reasons?
					☐ Yes ☐ No If yes, reason?
					☐ Yes ☐ No If yes, reason?
					☐ Yes ☐ No If yes, reason?
					☐ Yes ☐ No If yes, reason?
					☐ Yes ☐ No If yes, reason?

H. Did (_____) ever work at a job handling: (If so, mark box and record total number of years.)

- Paints and solvents? ☐ ____ Years
- Dry cleaner for clothes? ☐ ____ Years
- Gasoline and oils? ☐ ____ Years
- Asphalt and tar? ☐ ____ Years
- Creosote? ☐ ____ Years
- Dyes and stains? ☐ ____ Years
- Crop dusts and sprays? ☐ ____ Years
- Tobacco leaves? ☐ ____ Years
- Fluorescent and neon lights? ☐ ____ Years
- Asbestos? ☐ ____ Years
- X-Ray equipment? ☐ ____ Years

I. Did he ever work as:

- Sandblaster? ☐ ____ Years
- Welder? ☐ ____ Years
- Stone worker? ☐ ____ Years
- Miner? ☐ ____ Years
  - If yes, what mining? _____
- Metal worker? ☐ ____ Years
  - If yes, what metals? _____
- Fry Cook ☐ ____ Years
- Carpenter or sawmill worker? ☐ ____ Years
- Mechanic (any type)? ☐ ____ Years

J. Did he ever work in:

- Steel mill? ☐ ____ Years
- Grain elevator? ☐ ____ Years
- Road construction or maintenance? ☐ ____ Years
- Smelter? ☐ ____ Years
  - If yes, what metal? _____
- Any other dusty jobs? ☐ ____ Years
  - If yes, what jobs? _____

(Fig. 2 continued)

PART III
Cigarette Smoking Habits of the Deceased

A. As far as you know did (____) smoke as many as 5 to 10 packs of cigarettes during his entire lifetime?
☐ Yes If yes, | ☐ No If no—Skip to Part IV

B. During the 12 month period before death, did (____) smoke cigarettes?

1. ☐ Yes (Ask questions below)
   a. How many cigarettes did (____) smoke a day on the average during this period?
      1. ☐ Smoked once in a while, not every day
      2. ☐ Less than 10 cigarettes (less than ½ pack)
      3. ☐ Between 10 and 20 cigarettes (½–1 pack)
      4. ☐ Between 21 and 40 cigarettes (1–2 packs)
      5. ☐ Over 40 cigarettes (over 2 packs)
   b. Do you know when (____) started to smoke at this rate?
      1. ☐ Yes When? 19____
      2. ☐ No
   c. Before (____) smoked at this rate about how many cigarettes did he smoke on the average each day?
      ____ cigarettes

2. ☐ No (Ask questions below)
   a. Do you know when (____) stopped smoking?
      1. ☐ Yes When? 19____ (year)
      2. ☐ No
   b. Before (____) stopped smoking, about how many cigarettes did he smoke on the average each day?
      1. ☐ Smoked once in a while, not every day
      2. ☐ Less than 10 cigarettes (less than ½ pack)
      3. ☐ Between 10 and 20 cigarettes (½–1 pack)
      4. ☐ Between 21 and 40 cigarettes (1–2 packs)
      5. ☐ Over 40 cigarettes (over 2 packs)

C. Do you know how old (____) was when he first started to smoke cigarettes regularly?
1. ☐ Yes How old was (____)? _____years of age
2. ☐ No How old was (____) when you first knew that he smoked cigarettes? _____years of age

D. Did (____) ever stop smoking or change his smoking rate for health or medical reasons?
1. ☐ No 2. ☐ Yes If yes, how recently? 19____ (year) 3. ☐ Don't know

E. What was the largest number of cigarettes (____) ever smoked regularly each day?
1. ☐ Smoked once in a while, not every day
2. ☐ Less than 10 cigarettes (less than ½ pack)
3. ☐ Between 10 and 20 cigarettes (½–1 pack)
4. ☐ Between 21 and 40 cigarettes (1–2 packs)
5. ☐ Over 40 cigarettes (over 2 packs)

F. How long did (____) smoke at this rate? _____ (years)

PART IV
Cigar and Pipe Smoking Habits of the Deceased

A. Did (____) ever smoke any cigars?
1. ☐ No (Skip to question D)
2. ☐ Yes

B. Do you know how old he was when he started to smoke cigars?
1. ☐ Yes How old was he? ____ years of age
2. ☐ No How old was he when you first knew he smoked cigars? ____ years of age

C. What was the largest number of cigars he smoked regularly each day?
1. ☐ Smoked once in a while, not every day
2. ☐ 1 or 2 cigars
3. ☐ 3 or 4 cigars
4. ☐ 5 to 8 cigars
5. ☐ 9 or more cigars

D. Did (____) ever smoke a pipe?
1. ☐ No (skip to Part V) 2. ☐ Yes

E. Do you know how old he was when he started to smoke a pipe?
1. ☐ Yes How old was he? ____ years of age
2. ☐ No How old was he when you first knew he smoked a pipe? ____ years of age

F. What was the largest number of pipefuls he smoked regularly each day?
1. ☐ Smoked once in a while, not every day
2. ☐ Less than 5 pipefuls
3. ☐ Between 5 and 9 pipefuls
4. ☐ Between 10 and 19 pipefuls
5. ☐ 20 or more pipefuls

(Fig. 2 continued)

E. Was (____) ever more short of breath than most people his age when hurrying or walking up hills?
☐Yes ☐No ☐Don't Know If no or don't know, skip to Section F
If yes, then at what stage of his illness? ☐Beginning ☐Middle Stage ☐Last 12 months

	STAGES OF (____)'S ILLNESS								
	Beginning			Middle Stage			Last 12 Months		
	Yes	No	DK	Yes	No	DK	Yes	No	DK
Did (____) have to walk slower than other people his age because of shortness of breath?	☐	☐	☐	☐	☐	☐	☐	☐	☐
Did (____) ever have to stop for breath when walking at his own pace on level ground?	☐	☐	☐	☐	☐	☐	☐	☐	☐
Did (____) ever have to stop for breath after walking about 100 yards (or a few minutes) on level ground?	☐	☐	☐	☐	☐	☐	☐	☐	☐

F. Did (____) suffer from frequent colds? ☐ Yes ☐ No ☐ Don't Know
How many colds per year did (____) have at:
The beginning of his illness ____, during the middle stage ____, and during the last 12 months ____?

	Beginning			Middle Stage			Last 12 Months		
	Yes	No	DK	Yes	No	DK	Yes	No	DK
Did (____)'s colds seem to go down to his chest?	☐	☐	☐	☐	☐	☐	☐	☐	☐
Did (____) have a cough with his colds?	☐	☐	☐	☐	☐	☐	☐	☐	☐

G. When his illness first started when (____) was working regularly was he ever forced to stay at home because of breathing or respiratory difficulty?
☐Yes ☐No ☐Don't Know ☐Was not working regularly

If Yes:
1. For how long was (____) forced to stay home? _____
2. What was the job?_____
3. What time of the year was it?_____
4. Was the respiratory difficulty caused by a cold or was there a cold associated with it?
☐Caused by a cold
☐Cold associated with it
5. How often did it happen?_____

H. During the last year when (____) was working regularly was he ever forced to stay at home because of breathing or respiratory difficulty?
☐Yes ☐No ☐Don't Know ☐Was not working regularly (Skip to Section I)

If yes, then
1. For how long was (____) forced to stay home?_____
2. What was the job?_____
3. What time of year was it?_____
4. Was the respiratory difficulty caused by a cold or was there a cold associated with it?
☐Caused by a cold
☐Cold associated with it.
5. How often did it happen?_____

I. Between the time that (____) illness changed and the last 12 months when (____) was working regularly was he ever forced to stay home because of breathing or respiratory difficulty?
☐Yes ☐No ☐Don't Know ☐Was not working regularly
If yes: 1. For how long was (____) forced to stay home?_____
2. What was the job?_____
3. What time of year was it?_____
4. Was the respiratory difficulty caused by a cold or was there a cold associated with it?
☐Caused by a cold
☐Cold associated with it
5. How often did it happen?_____

J. Has any other member of your family immediate or otherwise ever suffered from symptoms similar to those (____) had?
☐Yes ☐No ☐Don't Know

(Fig. 2 continued)

PART V
Medical History of the Deceased

Now we would like to separate (——)'s respiratory illness into three stages: the beginning of his illness, the time between his illness becoming worse and the last 12 months of his life and finally, the last 12 months of his life.

A. When did (____)'s illness first start? ____ years ago

From the time the illness first started and the last 12 months of life did the (____) illness change?

☐Yes ☐No

If yes,

1. Was the change sudden? ☐Yes ☐No
2. Was the change gradual? ☐Yes ☐No

How long was the time between (____)'s first illness and the time that his illness changed?

____ Years ____ Months

What was the length of time between the change in (____)'s illness and the last 12 months?

____ Years ____ Months

B. Did (____) have any of the following?

Cough? ☐Yes ☐No ☐DK

Cigarette or smoker's cough? ☐Yes ☐No ☐DK

Coughing spells? ☐Yes ☐No ☐DK

Morning cough? ☐Yes ☐No ☐DK

If no or don't know, skip to Section D.

If yes to any of the above, at which stage of illness did it occur?

☐Beginning
☐Middle Stage
☐Last 12 Months

C.

		STAGES OF (____)'S ILLNESS		
		Beginning Check One Box	Middle Stage Check One Box	Last 12 Months Check One Box
How frequently did (____) cough?	Did not cough	☐	☐	☐
	Only when he had a cold	☐	☐	☐
	Less than 3 days a week	☐	☐	☐
	3 to 6 days a week	☐	☐	☐
	Some every day	☐	☐	☐
	A great deal every day	☐	☐	☐
	Don't know	☐	☐	☐
How severe was (____)'s cough?)	Did not cough	☐	☐	☐
	Required cough medicine	☐	☐	☐
	Often disturbed his sleep	☐	☐	☐
	Occurred in attacks or spasms	☐	☐	☐
	Was associated with breathlessness	☐	☐	☐
	Don't know	☐	☐	☐

D. Did (____) ever bring up any phlegm or sputum in the morning or at any other time of the day or night?

☐Yes ☐No ☐Don't Know

If no or don't know, skip to Section E.

If yes at any stage of illness, complete the following question:

		STAGES OF (____)'S ILLNESS		
		Beginning Check One Box	Middle Stage Check One Box	Last 12 Months Check One Box
What did it look like?	None	☐	☐	☐
	Scanty	☐	☐	☐
	White or mucous	☐	☐	☐
	Yellow or greenish	☐	☐	☐
	Occasionally bloody	☐	☐	☐
	Don't know	☐	☐	☐

FIG. 3. Questions asked of consultants for cases in which chronic obstructive respiratory disease was underlying cause of death.

Check Appropriate Box

( ) 1. Cause of death established by information collected and that cause of death is _____.

( ) 2. Cause of death cannot clearly be allocated to one specific disease but is clearly a chronic respiratory disease.

( ) 3. Chronic respiratory disease is not clearly the cause of death.

( ) 4. Chronic respiratory disease is *not* the cause of death.

( ) 5. Insufficient data entered on form to establish any cause of death.

Additional Remarks:

study, four questionnaires had not been returned: the one which was returned to the physician, the case referred by the certifying physician, and the two cases requiring a third questionnaire. It was thought that, with more sustained follow-up, these questionnaires would have been completed. Therefore, in summary, twenty of twenty-four questionnaires were completed in three weeks. Aside from scheduling the time of interview, there was little difficulty in seeing the physicians.

On interviewing the 5 preselected physicians from the mail questionnaire group of physicians, no significant differences were observed in comparing information collected by personal interview and by mail questionnaires. Again, a lack of sustained follow-up caused the delay; it was not inherent difficulties of the questionnaire design.

The majority of the physicians interviewed in both groups referred to their records as questions were asked. Several stated that they had recently reviewed their records. A few—less than 10 per cent—stated that there was no record or that the only record available was in a hospital or institution. Each interview took from ten to fifteen minutes. None of the physicians gave any indication that they were unwilling to give at least that much time.

Analysis and interpretation: There was no essential difference concerning criteria for selecting a given cause of death between the sample based on underlying causes compared with contributory causes of death. In the 7 cases of bronchopneumonia, it was found that these cases represented acute respiratory conditions without discernible preceding or underlying chronic respiratory disease. Hence, bronchopneumonia cases were not included in the second phase of the study. The criteria used by physicians were based primarily on information provided in questions 2 and 3 of the physician questionnaire.

These questions elicited the signs and symptoms associated with chronic obstructive ventilatory disease and a history of illness consistent with such diseases. The specific criteria which were used most frequently are indicated in table 5. It is indicated in table 5 that roentgenographic studies were also considered important, primarily in cases of pulmonary emphysema. When the physicians were asked which types of data they considered of importance in determining a cause of death (question 8) they also stated that the signs, symptoms, and history of illness were the important types of data. No specific group of symptoms was present in one diagnostic category and not in another.

The first 37 questionnaires to be completed were submitted to the panel of consultant chest physicians in two randomly divided groups. Two physicians were asked to comment on each questionnaire. The number of cases involved was too small for statistical evaluation. From table 7, it appears that the consultants agreed among themselves as to diagnosis more often than each one agreed with the physician who completed the questionnaire. The consultants' opinions of the adequacy of the information collected (table 8) indicate that the personal interview questionnaires were more adequate than the mail questionnaires. The numbers are not significant. The difference may represent an artifact since, with the personal interview group, the consultants dealt with the edited work of one person, the interviewer. With the mail questionnaire group, the consultants dealt with the multitude of ways physicians use to answer questions.

Evaluation of the next-of-kin questionnaires is in a more preliminary stage. Except for one refusal, the first 40 persons questioned were approached either by mail or personal interview without difficulty. In this one case, the physician

TABLE 5a

UNDERLYING CAUSE GROUP

TYPES OF DATA CONSIDERED PERTINENT BY PHYSICIANS IN MAKING DIAGNOSES OF CHRONIC RESPIRATORY DISEASE

	Total	Signs and Symptoms	History	Roentgenographic Studies	Laboratory Tests	Therapeutic Tests	Other*
Total	33†	26	17	16	4	5	13
Asthma	9	8	6	1	1	3	0
Chronic bronchitis	0	0	0	0	0	0	0
Other chronic interstitial pneumonia	5	4	2	4	0	0	0
Bronchiectasis	4	2	3	1	0	0	3
Pulmonary emphysema	15	12	6	10	3	2	10

* This category includes: bronchoscopy, pulmonary function tests, autopsy findings, electrocardiographic reports, and any other unclassified data.

† Seven cases of bronchopneumonia are not included (*see* text).

TABLE 5b

CONTRIBUTORY CAUSE GROUP

TYPES OF DATA CONSIDERED PERTINENT BY PHYSICIANS IN MAKING DIAGNOSES OF CHRONIC RESPIRATORY DISEASES

	Total Cases	Signs and Symptoms	History	Roentgenographic Studies	Laboratory Tests	Therapeutic Tests	Other*
Total	44	32	19	17	1	3	8
Asthma	14	10	8	5	0	2	3
Chronic bronchitis	3	1	1	1	0	0	0
Other chronic interstitial pneumonia	2	1	1	2	0	0	1
Bronchiectasis	4	3	1	1	0	0	0
Pulmonary emphysema	21	17	8	8	1	1	4

* This category includes bronchoscopy, pulmonary function tests, autopsy findings, electrocardiographic reports, and any other unclassified data.

was interviewed again, and he said he should have advised against an interview with the wife of the decedent. Bereavement in the other cases did not seem to be a handicap. Each personal interview required about one hour, and the personal interviews were not significantly more complete than the mail interviews.

DISCUSSION

The follow-back approach, while not new (5) had never been tried with the chronic obstructive respiratory diseases under study. The technique appears to be adequate to determine the criteria physicians use in diagnosing these diseases. The follow-back approach may provide new opportunities for epidemiologic studies of respiratory disease. First, the technique may determine whether the sharp rise of deaths from these diseases is more apparent than real. By learning through repeated studies whether changes occur in the criteria physicians are using, it may be possible to determine whether diagnostic practices are changing or if the true incidence of these diseases is increasing. Second, the findings suggest that these diseases cannot be completely separated from each other. If, by determining the diagnostic criteria physicians use, it is found that they do not really separate these diseases one from the other, then—at least for statistical and epidemiologic purposes—the whole group of diseases can be considered together. Goldsmith (1) has discussed the epidemiologic implications of this possibility. Third, the influence of environmental exposures, such as air pollution, occupation, and smoking, may be studied by follow-back methods, thus facilitating the prevention of obstructive ventilatory diseases.

TABLE 6a

UNDERLYING CAUSE GROUP

CRITERIA USED BY PHYSICIANS IN MORE THAN 50 PER CENT OF CASES

	Total Cases	Cough	Sputum	Wheezing	Increased Anterior-Posterior Diameter	Dyspnea	Mobility of Diaphragm	Rales	History of Previous Illness
Total	33*	27	19	21	26	28	22	20	24
Asthma	9	7	6	7	7	7	7	7	5
Chronic bronchitis	0	0	0	0	0	0	0	0	0
Other chronic interstitial pneumonia	5	5	2	2	4	3	2	1	4
Bronchiectasis	4	3	2	2	2	3	3	4	4
Pulmonary emphysema	15	12	9	10	13	15	10	8	11

* Seven cases of bronchopneumonia are not included (*see* text).

TABLE 6b

CONTRIBUTORY CAUSE GROUP

CRITERIA USED BY PHYSICIANS IN MORE THAN 50 PER CENT OF CASES

	Total Cases	Cough	Sputum	Wheezing	Increased Anterior-Posterior Diameter	Dyspnea	Rales	History of Illnesses
Total	44	37	27	30	28	37	30	29
Asthma	14	10	7	14	7	13	9	11
Chronic bronchitis	3	3	3	1	1	1	2	2
Other chronic interstitial pneumonia	2	2	0	2	1	2	1	1
Bronchiectasis	4	3	2	2	2	3	4	4
Pulmonary emphysema	21	19	15	11	17	18	14	11

This pilot study showed no apparent difference between the criteria physicians use for assigning chronic obstructive respiratory diseases as underlying causes of death and the criteria used in assigning them as contributory causes of death. Also, it showed that, as far as the physicians were concerned, the same wording of questions may be used to study both underlying cause groups and contributory cause groups.

In addition to performing the functions of a pilot study, the writers believe that the less expensive mail questionnaire could be used effectively. It is apparent from this study that about as much information can be obtained by mail questionnaire as by personal interview. This makes it possible to carry out a large-scale study at a reasonable cost.

SUMMARY

A technique to obtain additional information about people who die from a specific group of

TABLE 7

DIAGNOSIS BY CHEST CONSULTANTS

	Total Cases Evaluated	Diagnosis Same as Diagnosis by Individual Physician	Diagnosis Different from Diagnosis by Individual Physician	Diagnosis not Determined by Consulting Physician
Consultant A	18	4	5	9
Consultant B	18	4	3	11
*Consultants A and B	18	3	4	6
Consultant C	19	8	4	7
Consultant D	19	8	2	9
*Consultants C and D	19	6	2	3

* Consultants A and B saw the same cases; Consultants C and D saw the same cases. Therefore, the row marked "Consultants A and B" and the row marked "C and D" indicate the number of cases in which the consultants agreed with themselves and either agreed or disagreed with the case physician, or did not determine diagnosis.

TABLE 8

COMPLETENESS OF QUESTIONNAIRE AS DETERMINED BY CONSULTANTS

	Consultant A			Consultant B			Consultant C			Consultant D		
	Total	Mail Questionnaire	Personal Interview	Total	Mail Questionnaire	Personal Interview	Total	Mail Questionnaire	Personal Interview	Total	Mail Questionnaire	Personal Interview
Total	18	7	11	18	7	11	19	9	10	19	9	10
1. Cause of death established	11	4	7	8	1	7	8	3	5	15	6	9
2. Cause of death not clear but is a chronic respiratory disease	2	1	1	6	3	3	2	0	2	0	0	0
3. Chronic respiratory disease not clearly cause of death	3	2	1	1	1	0	1	0	1	2	1	1
4. Chronic respiratory disease *not* cause of death	0	0	0	0	0	0	0	0	0	1	1	0
5. Insufficient data to establish any cause of death	2	0	2	3	2	1	8	6	2	1	0	1

chronic obstructive respiratory diseases has been designed. These diseases include asthma, chronic bronchitis, bronchiectasis, pulmonary fibrosis, and pulmonary emphysema. A comparison of the efficiency of mail versus personal interview methods was made. Physicians were interviewed to determine the diagnostic criteria used, and the next of kin were interviewed to determine what role environmental factors may have played in the course of a decedent's pulmonary disease. From the results of this pilot study, it appears that about as much information from physicians and next of kin can be collected by mail query as by personal interview.

SUMARIO

Estudios Epidemiológicos de la Enfermedad Ventiladora Oclusiva del Pulmón. II. Aborde de Retro-Observación para Estudiar la Mortalidad Debida a las Enfermedades Ventiladoras Oclusivas del Pulmón: Resultados de un Estudio Explorador

Se ha elaborado una técnica para obtener mayor información acerca de las personas que fallecen de un grupo específico de enfermedades oclusivas crónicas del aparato respiradorio. Comprenden estas enfermedades asma, bronquitis crónica, bronquiectasia, fibrosis pulmonar y enfisema pulmonar. Se hizo una comparación de la eficacia de los métodos de correspondencia y de entrevista personal. Se interrogó a los médicos para determinar las pautas de diagnóstico y a los allegados para determinar qué papel pudieron haber desempeñado los factores del ambiente en la evolución de la enfermedad pulmonar del fallecido. Por los resultados de este estudio parece que puede acopiarse más o menos tanta información de parte de los médicos y los allegados por encuestas por correo como con entrevistas personales.

RESUME

Etudes épidémiologiques de maladie ventilatoire obstructive du poumon. II. Un moyen d'étude pour suivre rétrorespectivement la mortalité des maladies ventilatoires obstructives du poumon: résultats d'une étude-pilote

Les auteurs ont inventé une technique pour obtenir des informations supplémentaires sur les personnes qui meurent d'un groupe spécial de maladies chroniques respiratoires obstructives. Ces maladies comprennent l'asthme, la bronchite chronique, les bronchiectasies, la fibrose pulmonaire et l'emphysème pulmonaire. On a comparé l'efficacité de la correspondance aux méthodes d'entrevue personnelle. On demanda aux médecins de déterminer les critères de diagnostic utilisés, et le plus proche parent fut interrogé pour déterminer quel rôle ont pu jouer les facteurs de milieu dans l'évolution de la maladie pulmonaire du décédé. D'après les résultats de cette étude-pilote, apparait que l'on peut obtenir à peu près autant d'information des médecins et du plus proche parent par demande écrite, que par entrevue personnelle.

Acknowledgment

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THE EXPIRATORY SPIROGRAM[1]

HOWARD DAYMAN

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Introduction

To warrant diagnostic acceptance, the expiratory spirogram should exhibit a reproducible configuration which reflects the underlying physical state of the respiratory tract. Justification for its use has depended mainly on inspection of the tracings and on the appraisal of certain gross indices of flow or volume. A few studies have suggested that certain aspects are reproducible or related to specific features of bronchopulmonary dynamics. The present writer has observed that the abrupt decline of flow in emphysema was related to the check-valve mechanism (1). Pierce (2) found that outflow in health followed much the same course whether expiration was passive or forced, despite a tenfold difference in pressure. Hyatt (3) reports that the first half of expiration is effort dependent and variable, while the last half adheres to a consistent pattern with rate of flow linked to volume.

The purpose of the present study is to test the diagnostic validity of the spirogram through detailed analysis of flow during a forced vital capacity expiration. Normal limits are determined and departures therefrom are defined in a large number of patients suffering from ventilatory disorders. Cases are classified by disease and are further grouped according to the primary mechanical derangement which, it is found, exerts a dominant influence on the character of the spirogram. Categories include: restriction of the volume of displaceable air, intrinsic airway obstruction, decreased lung tension with respect to the degree of inflation, and the opposite condition of increased lung tension.

There are uncertainties which beset analysis of naturally occurring clinical phenomena. Action of one factor cannot be divorced from other factors caused by complications or associated disease, short of experimentally creating the isolated condition in an otherwise healthy human subject. This would be a difficult undertaking for most of the factors of interest in this study. However, the cause of an observed effect may often be postulated by invoking current knowledge of respiratory mechanics, supported, when necessary, by confirmatory physical measurements. Factors which cannot be isolated may still be altered to permit comparison. Among these are force, volume, lung tension through change of volume, and bronchospasm under the influence of adrenalin-like medication. Findings derived by such means have suggested a general concept of ventilatory mechanics which has been found useful in diagnosis.

Material and Methods

Method: A 6-liter spirometer was modified for the purpose. A 4 r.p.m. motor gave the record a surface speed of 32 mm. per second. The subject breathed through 10 inches of brass tubing, 1.5 inches in diameter, the end of which was squeezed to ensure a 1-inch gap between the teeth and flanged to seal behind the lips. Thus modified, the device will record the ebb and flow of 4 cc. of air fourteen times a second. Capacity for such rapid response gives credence to the indications of sudden change of flow rate in the spirogram.

An alternate instrument was the pneumotachograph, convenient for other breathing maneuvers, such as successive coughs. It comprised the usual screened breathing tube tapped for gradient across the screen, a P97 strain-gauge transducer, and Sanborn amplifier/recorder. Response was linear up to about 8 liters per second, the range of greatest interest. To secure the same prompt response to flow in either direction, it was found advisable to include a small chamber in the tubing going to one side of the diaphragm to equalize volume on either side. The tubing was likewise passed through squeeze blocks so that flow resistance to either side of the diaphragm could be equalized. Resistance of the screen varied from time to time, so a breath was blown through the PTG tube into the spirometer which could be expected to record volume accurately. If the integrated PTG curve did not give a volume which matched that of the spirometer, a correction factor was introduced. Patterns of flow for both instruments compared so well that they are used interchangeably in this report. Maximal breathing capacity was measured with the spirometer. For typical subjects of each group, lung tension was measured through the range of vital capacity by a simplified method which, with a little practice, gave results comparable to those recorded by a strain gauge. During brief interruption of flow, the difference of pressure between the mouth and

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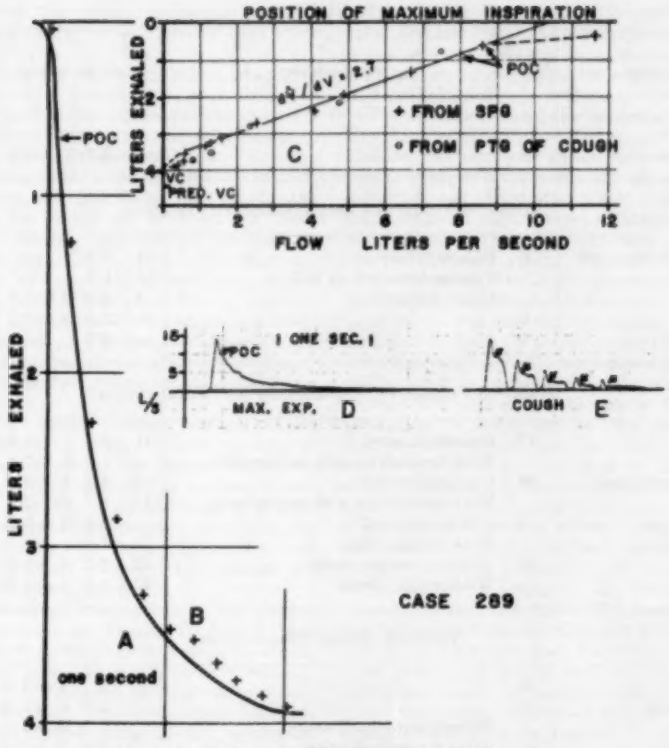

FIG. 1. Expiratory flow in the normal adult male:
A: Solid line is the spirogram traced with the spirometer.
B: Points marked + were plotted by integrating the pneumotachogram of *D*.
C: The points marked + are from the spirogram (SPG) of *A*. Critical flow plotted against volume exhaled gives a straight line. The three parts of the spirogram are shown, the initial effort-dependent part, the period of critical flow, and the small terminal part. At transition from critical flow to terminal flow a change in rate of deceleration creates a small "hook" in the plot of flow against volume. This is uniformly present, cause unknown. Points marked *o* are plotted from the succession of coughs in *E*.
D: Pneumotachograph of a maximal expiration.
E: Pneumotachograph of a succession of coughs.

esophagus was read on a small-bore cistern type of manometer.

Patients were asked to start the exhalation energetically, so peak flow was reached almost immediately and was identified as the point having the most acute angle with the vertical. If a tangent to this point is extended upward until it intersects a transverse line at the level of zero volume, the intersection provides a fairly standard point of departure for timing the spirogram. Any method would be arbitrary. This one assumes that acceleration of flow is instantaneous. From the slope of line, peak flow and flow at tenth-second intervals were measured and plotted against volume on a separate graph which accentuates features which would otherwise be overlooked. The same plot can be made from the pneumotachograph tracing of a maximal expiration or for a series of identifiable points in a succession of coughs. The results with all methods are illustrated in figure 1.

Clinical material: The conditions studied are listed in table 1. Forty-eight subjects were normal in their denial of respiratory symptoms or disease. The remainder were patients seeking relief of symptoms or offering them as a basis for pension. The patients received complete histories and physical examinations, chest roentgenograms or fluoroscopies, and additional tests when indicated.

The Normal Spirogram

Descriptive features: Essential elements of one spirogram can be found in all others. Differences

TABLE 1

Clinical Goup	Number	Clinical Subgroup	Number	$d\dot{V}/dV$	Range	Useful Volume	Maximal Critical Flow
						liters	*L./sec.*
Normal	48	—	—	2.7	1.7–3.9	3.2	9.1
Reduced Volume of Displaceable Air: Bellows							
Weak or ineffective bellows	6	Poliomyelitis	1	2.5	—	2.5	6.8
		Transected cord at C-7	1	1.7	—	0.9	2.0
		Chest deformity	4	3.0	2.6–3.1	1.7	5.4
Obesity	5	—	—	2.5	2.0–2.7	2.8	7.7
Pregnancy	1	—	—	2.9	—	1.6	5.0
Reduced Volume of Displaceable Air: Parenchyma							
Ablation	17	Uncomplicated	11	2.7	1.7–3.6	1.7	4.8
		With bronchitis and emphysema	6	1.4	1.0–2.1	2.0	3.2
Inactive tuberculosis	33	Uncomplicated	18	2.2	1.2–2.7	2.1	4.6
		With bronchitis and emphysema	15	1.7	0.7–2.1	1.9	3.2
Boeck's sarcoid	3	Uncomplicated	2	2.6	2.4–2.8	2.2	6.2
		With emphysema	1	1.4	—	1.0	1.4
Silicosis	27	Without emphysema	12	2.2	1.2–2.8	2.2	4.9
		With emphysema	15	1.6	0.6–2.5	1.7	2.6
Intrinsic Airway Obstruction							
Bronchitis	25	—	—	1.7	1.0–3.0	2.4	4.3
Asthma, active	17	—	—	1.0	0.5–1.8	2.0	2.6
		Before bronchodilators	8	0.9	0.5–1.6	1.4	1.7
		After bronchodilators		2.0	1.7–2.8	2.2	4.9
Asthma, natural remission	13	—	—	2.1	1.4–3.3	2.1	4.9
Reduced Lung Tension, Emphysema Group							
Emphysematous bullae	7	No bronchitis	3	2.4	2.2–2.8	2.2	6.4
		Bronchitis before bronchodilators	4	1.2	0.8–1.7	1.9	2.8
		Bronchitis after bronchodilators		1.5	1.0–2.3	2.4	4.1
Anatomic emphysema, dry generalized	33	$d\dot{V}/dV$ measurable	15	1.5	0.8–4.0	0.7	1.4
		Not measurable	18	—	—	0.7	—
Anatomic emphysema with bronchitis or asthma	45	—	—	1.2	0.3–2.6	1.2	2.2
		Before bronchodilators	14	1.0	0.3–1.7	1.1	1.8
		After bronchodilators		1.6	1.1–2.5	1.4	2.7
Increased Lung Tension							
Interstitial pneumonitis	15	Uncomplicated	12	4.9	3.0–14.0	1.8	8.4
		With emphysema	3	2.0	1.4–2.6	1.4	3.2
Other	31	—	—	—	—	—	—
Total	326						

are quantitative. The normal spirogram provides a basis for reference. It is divisible into three parts (figure 1*C*).

The first part, the effort-dependent phase, includes the period of acceleration, peak flow, and the very first part of the deceleration to the point at which deceleration becomes abruptly slower to mark the beginning of the second phase. Duration of the first part is found to be less than 0.1 second; and the volume, less than 1 liter. The greater the initial effort, the higher the peak of flow; and, within certain limits, the smaller the volume. Expiratory restraint can protract the effort-dependent phase to the extent described by Hyatt (3). The margin by which peak flow of the first phase exceeds that of the second phase is a criterion of adequate effort. The deeper the inspiratory position at which expiration is initiated, the larger the volume (all else being constant) and the higher the peak of flow.

Plotting flow against volume brings out the abrupt assumption of a slower uniform rate of deceleration which marks the transition to the second phase. Cough entails the highest initial pressure and magnifies the misalignment of flow between the phases. In a succession of coughs, the junctures are marked by darts (figure 1*E*), and toward the expiratory position the tracings are not only angulated but notched.

These phenomena are implicit in the check-valve mechanism described earlier (1, 4, 5). Within the airways during flow, pressure declines downstream, but the airways are subjected externally to pressure of the enclosing thoracic space. Compression narrows them. This brief period of adjustment delimits the first phase of the spirogram (SPG) and its completion is designated point of closure (POC) (figure 1). Glottic obstruction restores the airways to static size determined by the volume of the lungs, mediated through lung tension. Each cough starts with a new effort-dependent phase. Greater expiratory effort hastens the adjustment, elevates peak flow, and limits volume. It can momentarily compress the airways too much, whereupon they rebound—the notch in the cough tracing—or flutter if the process is repetitive. The last-named manifestations are prone to occur when resistance in upstream airways is high, physiologically near the expiratory position, and pathologically in asthma or emphysema.

The second part of the spirogram, designated the phase of critical flow, begins with the point of closure. The terminus varies somewhat from subject to subject, but error is not serious if it is considered to end at the place where flow decreases to 0.5 liters per second. There, an abrupt momentary decrease of deceleration imparts a small hook to the plot for flow against volume. Flow is called critical because flow is not altered when the subject exceeds the threshold of pressure necessary to produce it. The pattern for successive coughs is identical with that of uninterrupted forced expiration (figure 1*C* and *E*). Expiratory restraint protracts the first phase, delaying the emergence of critical flow. But since the requisite pressure for producing it decreases as the lungs become smaller, critical flow does ultimately appear. Restraint rendered the first half of the spirogram effort-dependent in Hyatt's study (3). At a given point in the vital capacity, critical flow is fixed regardless of excessive effort. This is one of the two essential characteristics of critical flow.

The second is a uniform rate of deceleration with respect to volume, in healthy subjects amounting to a decrease of 2.7 liters per second for every liter exhaled. Flow plotted against volume gives a straight line. Volume plotted against time gives a *J*-shaped exponential curve, the spirogram itself. In healthy subjects the check-valve mechanism regulates flow instead of virtually arresting it, as happens in advanced emphysema. A check-valve properly spring-weighted against closure becomes a flow-control valve. Human airways embody the necessary elements. Large downstream airways, those subjected to the greatest compression, are equipped with cartilaginous arcs completed posteriorly by a membrane. This invaginates under external pressure, stressing the cartilage which is designed to offer graded resistance to deformation. Pressure difference be-

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This table describes salient aspects of flow for diseases classified according to the physical abnormality of lung on which the principal spirographic feature depends, the primary mechanical derangement. Conditions may limit displaceable volume by direct interference with bellows excursion, or may do so indirectly by reducing the volume of air available for displacement. These are different mechanisms, but they produce the same result in the spirogram because a large amount of lung inflated to less than maximal volume has the same outflow pattern as a small amount of lung fully inflated to the same volume. Likewise, there are two mechanisms for intrinsic obstruction, bronchospasm and secretions, but clinical designations do not lend themselves to their separation. Intrinsic obstruction is distinct from that due to loss of lung tension through parenchymal breakdown. The former is responsive to bronchodilators while the latter is fixed. Although increased lung tension reduces displaceable volume, the salient spirographic features are concerned with flow; hence, it falls in a separate group.

The group marked "other" is included for completeness. Some subjects were not clinically classifiable. In others there were cardiac conditions, the airflow characteristics of which are at times difficult to explain.

Frequency of complications emphasizes the difficulty in obtaining conditions in pure form for study. Even when a single condition is designated, complicating disease was doubtless present in some degree even though it might be in occult form.

tween the outside and the inside of the airway, and hence the degree of narrowing, depends on the canalicular pressure gradient between the downstream airways and the upstream terminal spaces which surround them. Greater effort, by accentuating the gradient, further narrows the downstream airways, increasing their resistance to flow, which thereby becomes fixed at a level predetermined for that position in the vital capacity. But the gradient also varies with resistance to flow in upstream airways, the bronchioles for example. For these, location minimizes check-valve compression. Diameter is determined by the static pressure of lung tension. Hence, the level at which flow is fixed becomes progressively lower as the exhalation proceeds. Rate of critical flow varies inversely with the volume of inflation.

The first and second parts, together, comprise the useful volume. A small terminal part completes the vital capacity of which it comprises approximately 11 per cent, or roughly half a liter in the healthy subject. In this part, flow is too slow to be physiologically useful. It must be considered separately for several reasons. Flow tends to be erratic in the terminal portion, unlike the preceding critical flow. In advanced emphysema it makes up more than half the vital capacity. In the present experiments, transition from the second to the third part of the vital capacity takes place at the point at which lung tension becomes zero. The terminal part seems uniquely to be extruded at slow leakage flow under conditions of depleted lung tension.

Quantitative aspects: Critical flow from normal lung is distinguished by an unvarying value for change of flow with respect to change of volume ($d\dot{V}/dV$). In 48 normal subjects, 24 of them male, it was 2.7 ± 0.4 S.D., range 1.7 to 3.9 (*see* table 1). The great majority, 42 in number, fell within the range 2.2 to 2.9. The normal subjects comprised a random sample of those claiming to be healthy, but some may well have harbored some occult disorder of airways or parenchyma. In every instance, 72 per cent or more of the vital capacity was expelled in the first second. Vital capacities averaged 97 per cent of the values predicted by the equation of West (6). Breathing capacities were within normal range by the standards of Baldwin (7). Values for $d\dot{V}/dV$ did not significantly vary for males and females. Stature did not affect it. The value was the same for 4 children. Finally, the lung remaining after surgical ablation, providing it was healthy, gave the same value (*see* table 1). Lungs seem to be made up of identical functional units each bearing a constant relation between capacity for outflow and the state of inflation. Multiples thereof retain the same relation. Larger subjects simply have a larger number of participating units.

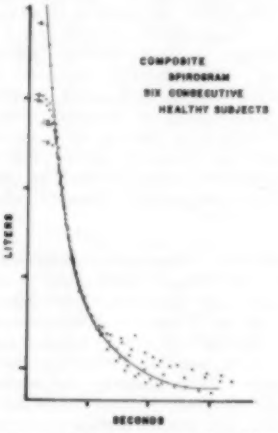

FIG. 2. Four of the 6 normal subjects were women. The series of dots trace the spirogram. Note the three parts: First, an introductory phase; then critical flow which is identical for all 6 subjects: then a variable terminal portion. Except for the small initial and terminal portions, normal spirograms have one unvarying configuration, differing only in length.

To illustrate these findings and to emphasize the consistency with which they occur, 6 consecutive visitors to the laboratory were asked to trace spirograms. They claimed to be healthy, were untutored in spirometry, varied in stature, 4 of them were females. The course of each spirogram is shown as dots in figure 2, together with a line which traces the curve defined by the equation $d\dot{V}/dV = 2.7$. In the second phase, that of critical flow, virtually all of the dots and the line can be effaced by a single stroke of the pen. Variability of the third phase is well shown and attests the need to deal with terminal flow separately.

A few additional observations warrant description. In the presence of obstructive disease, excessive effort may reduce flow equally at all points, but the rate of deceleration is not affected. If effort is withheld so that expiration is restrained, it protracts the first phase, delaying the emergence of critical flow. Laryngeal narrowing reduces flow by a different mechanism. It lowers the canalicular gradients which govern airway diameter. Inserted midway in the breath this hastens deceleration and the change makes it detectable. If laryngeal narrowing takes over control of flow early in the breath, it may not be possible to determine the reason for the high value for $d\dot{V}/dV$.

If flow and the constant for its deceleration are known, it should be possible to compute quantitative values for certain static properties of the lungs. This involves variables about which little is known except in healthy subjects. *Case R.W.* (figure 3) illustrates how elastic tension of the lungs can be determined from flow.

When vital capacities vary between 0.5 liters and 5.0 liters and the value for $d\dot{V}/dV$ is 2.7, the portion exhaled in the first second is remarkably constant, range 81 to 88 per cent of the total. The one-second expiratory capacity often corresponds

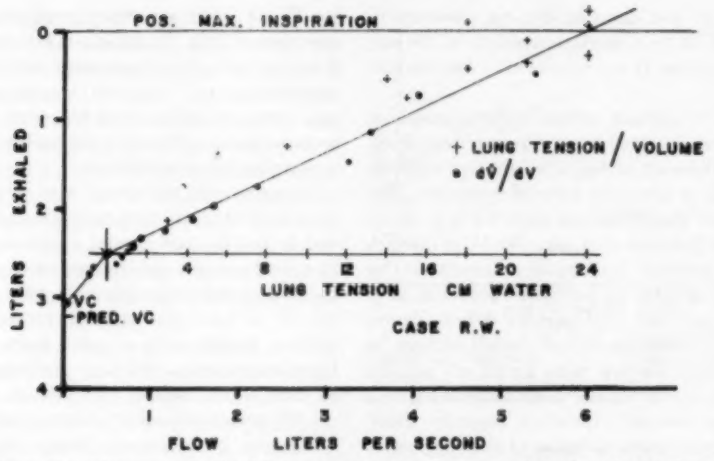

FIG. 3. From the spirogram of a healthy young woman, flow was plotted against volume, the dots (●). Value for $d\dot{V}/dV$ was 2.2. With the aid of an esophageal balloon, transpleural pressures were also measured over the range of the vital capacity. Lung compliance was 0.10 liters per cc. of pressure change, a somewhat low figure, but the subject was a small woman. By using the proper scale for lung tension, both compliance and $d\dot{V}/dV$ can be indicated by the slope of one line. Both tension and flow are directly related to volume. Knowing both $d\dot{V}/dV$ and lung compliance, tension can be computed from flow, and the reverse. An important value is maximal lung tension, in this case 24 cm. of water pressure. It has been observed to range in healthy subjects between 20 and 28 cm. Fry (8) reports similar figures. In the *Handbook of Respiration* (9), compiled data on compliance indicate a mean value of 23 cm. for maximal lung tension.

closely to the summated volume of the first two phases which is called useful volume.

Maximal critical flow, obtained by extending the line for $d\dot{V}/dV$ to the position of maximal inspiration, is a theoretic value which assumes that the adjustment of the effort-dependent phase took place instantaneously.

The Expiratory Spirogram in Disease

Terms employed to describe the normal spirogram apply equally well to the spirogram in disease. All exhibit the same features, differences being those of degree. The changes in volume are shown in figure 4; changes in flow, in table 1.

Restriction of the volume of displaceable air: These otherwise disparate diseases share a common primary mechanical derangement brought about by direct interference with bellows movement, by space filling, by excluding part of the parenchyma from ventilation, or by a combination of these means. Whatever the cause, inspiratory displacement is curtailed. Even though a space-filling lesion (such as pneumothorax) limits expiratory excursion of the bellows, the effect on ventilatable parenchyma is to extend expiratory displacement and limit inspiration.

Surgical ablation and exudative, granulomatous, fibrotic, or atelectatic foci exclude parenchyma from ventilation. In ablation cases, it was found that the retained volume corresponded approximately to the proportion of tissue remaining, and the flow/volume relation was normal. It was normal or nearly so in uncomplicated tuberculosis, silicosis, and Boeck's sarcoid. The stage of disease affected displaceable volume but not $d\dot{V}/dV$. Patients in whom the breathing bellows was restricted retained a normal flow/volume relation. In one patient, severance of the spinal cord at the seventh cervical vertebra resulted in paralysis of all muscles involved in respiration except the diaphragm. Expiration had to be passive, yet outflow followed a course not far removed from normal.

Diseases which primarily limit the volume of displaceable air cause proportionate reduction of flow without changing the flow/volume relation. The amount of restriction parallels the severity of disease. Cause for the restriction cannot be determined from the spirogram. The per cent of the test vital capacity exhaled in the first second remains normal.

Intrinsic obstruction of the airways: Intrinsic ob-

struction of the airways denotes obstruction brought about by a morbid condition of the airways themselves. It is represented by asthma and bronchitis.

Asthma: Important criteria in the diagnosis of asthma in these subjects were paroxysmal dyspnea with wheezing, allergic etiology, and capacity for natural or adrenalin-induced remission. The spirographic manifestations included low maximal critical flow and a low value for $d\dot{V}/dV$, which could be restored to normal or nearly so by adrenalin (*see* table 1), premature termination of useful volume, and vital capacity deficit (figures 4 and 5.4). Adrenalin caused a small increase in useful volume. The low value for $d\dot{V}/dV$ and the extended terminal volume both contributed to a reduced one-second expiratory capacity which was a discriminatory indicator of the response to adrenalin.

If spasm of the bronchioles accompanies asthma, the foregoing concept of mechanics offers a ready explanation for spirographic manifestations. Spasm reduces the over-all diameter of bronchioles and reduces the change of diameter which can be brought about by a unit change in ung tension. These result in low critical flow and low $d\dot{V}/dV$. Narrow airways terminate useful volume prematurely. In asthma it represents something less than the volume range over which lung tension is exerted. Enlarged terminal volume and vital capacity deficit persisted after the use of bronchodilators. These can reasonably be ascribed to complicating emphysema.

Bronchitis: In the group with bronchitis the symptoms of obstruction were usually less severe and lacked the paroxysmal qualities of asthma. In some there were manifestations of allergy, and the flow characteristics, including the response of $d\dot{V}/dV$ to bronchodilators, resembled those of asthma. In others, as occurred more frequently, bronchodilators caused a proportionate increase in both useful volume and rate of flow, while $d\dot{V}/dV$ remained fixed at pretreatment level.

Whereas bronchospasm causes reversible reduction of $d\dot{V}/dV$, obstructing secretions primarily reduce useful volume by excluding portions of parenchyma from ventilation. Value for $d\dot{V}/dV$ in bronchitis is variable, depending on existing complications, concurrent bronchospasm and emphysema, both of which depress $d\dot{V}/dV$. Lacking these, as in the instance cited in figure 5*B* (*Case J.H.*), the value for $d\dot{V}/dV$ is normal.

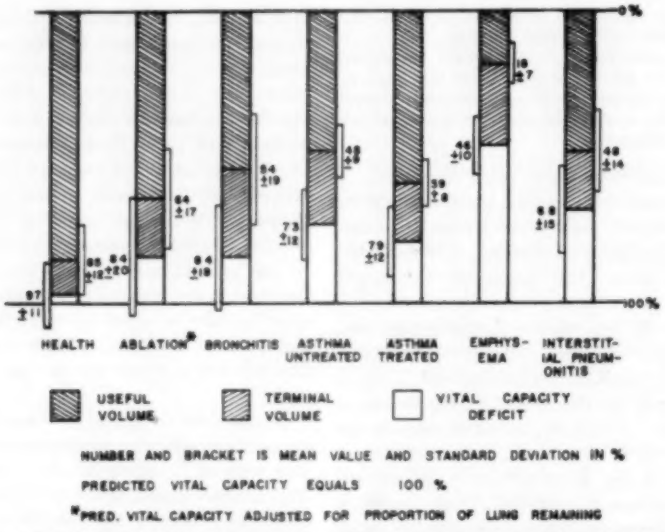

FIG. 4. Important volumes in the spirogram are useful volume, which combines the effort-dependent phase and the phase of critical flow, and the terminal volume. On either side of the bars in the graph are brackets and numerals which show the standard deviation from the mean for useful volume and vital capacity. Vital capacity was predicted by the equation of West (6) without correction for degenerative lung diseases which attend aging.

Low lung tension—the emphysema group: As here employed, the term emphysema denotes the form characterized by breakdown of alveolar walls and coalescence of spaces. In most cases the disease was advanced; the breathing capacity was markedly reduced; and dyspnea, prohibitive if exertion exceeded that of slow walk. Breath sounds were absent over wide areas. Expiratory effort met with early abrupt slowing. Thin rims of bullae were outlined on the roentgenogram. The condition responded poorly or not at all to adrenalin and was unremitting in the subsequent natural history of the disease.

Bullous emphysema: In the subjects with bullous emphysema, areas of parenchymal dehiscence were visibly and audibly set apart from relatively intact areas of better or even good function. Flow characteristics are shown in table 1: In 3 uncomplicated cases of slight bulla formation, reduced useful volume was the only change. Bronchitis was present in cases of advanced disease.

Findings in *Case R.L.* (figure 6) are typical. Exhalation took place in sequence from two distinct and identifiable lung compartments. First, the more intact areas exhaled at relatively rapid rates of flow. Whereas $d\dot{V}/dV$ was normal in slight cases, it was low and fixed in those of advanced disease. Response to bronchodilators was of the type associated with expulsion of secretions. Emphysema itself, if sufficiently advanced, can depress $d\dot{V}/dV$, independent of the action of bronchospasm. Exhalation from emphysematous areas followed. It was slow and incomplete, causing a vital capacity deficit. Transition from useful volume to terminal volume on the spirogram marked cessation of flow from relatively intact parenchyma and its continuance from emphysematous parenchyma. Useful volume reflects the volume range over which lung tension is exerted by the intact parenchyma and necessarily varies with the amount of such parenchyma participating in exhalation.

Anatomic emphysema, generalized, dry: The expression implies structural abnormality of the parenchyma, with signs and symptoms of bronchitis or asthma few or absent. Generalized in the sense of being widely dispersed, some areas are more severely affected than others, two compartments as in the previous group. Ventilatory disparity between one part and another is dramatically demonstrated by ventilating emphysematous lungs outside the body. Rapid cycling virtually eliminates participation of the more emphysematous portions (4, 5). It may not be possible to detect lines of demarcation clinically; more often than not the breath sounds and fluoroscopic findings disclose zones where ventilation distinctly differs from that prevailing elsewhere.

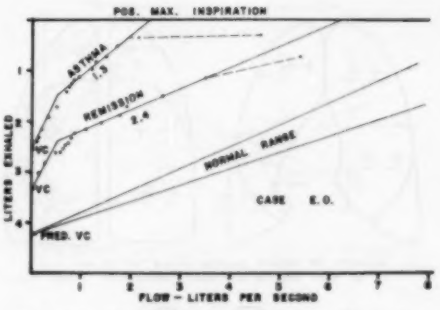

FIG. 5*A*. *Case E.O.* Had allergic asthma from industrial inhalants. Leave from work brought remission. The change in $d\dot{V}/dV$ is characteristic for the allergic asthma group.

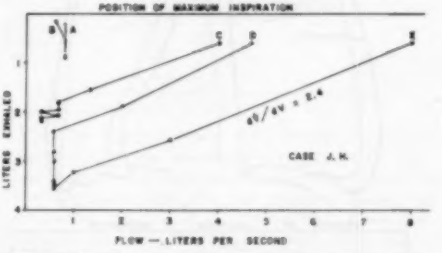

FIG. 5*B*. *Case J.H.* had an unusually severe bronchitis. Life was threatened by thick tenacious mucus which could not be effectively displaced by the usual means (series A and B). With the aid of bronchoscopy and direct aspiration, progressive improvement occurred. Series C–E trace the improvement of breathing, but $d\dot{V}/dV$ remained within normal limits. The changes of flow and volume are equivalent when secretions exclude parts of the parenchyma from ventilation, unlike cases of bronchospasm wherein flow is predominantly affected.

The effort-dependent part of the spirogram is small, but peak flow is likely to be good (five liters per second or more) if exhalation is energetic, in contrast to the ensuing low rate of critical flow. If disease is advanced, the latter may entail so little volume that the course of $d\dot{V}/dV$ cannot be determined. If determinable, the value is low

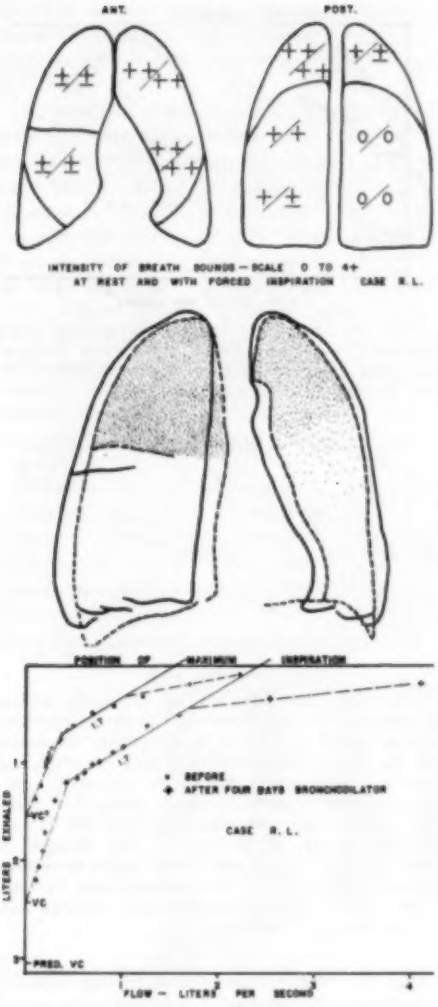

FIG. 6A (Upper). Plus signs indicate the intensity of the vesicular murmur at rest and with forced inspiration, on a scale of 0 to 4 plus. Normal would be plus/minus to three plus. Ventilation was poor in the left lower lobe, absent in the right lower, poor in the right middle and right upper. The best breathing was in the left upper lobe, but there was no reserve. *B* (Center). The outlines were taken from inspiration film (solid line) and expiration film (dashed line). The density of the stippling indicates the density of the lung roentgenogram in expiration. Both bases compressed the upper portions on expiration. The left base exhaled slowly; the right, not at all. Note the correlation with physical signs. *C* (Lower). Expiratory flow plotted against volume exhaled. Numerals refer to the value for $d\dot{V}/dV$. The change brought about by bronchodilators indicated release of secretions rather than release of bronchospasm. The three parts of the spirogram are well shown. The first two, the effort-dependent phase and the period of critical flow, comprise the useful volume. This represents outflow from the relatively intact parenchyma. Thereafter, air leaks slowly from the emphysematous part, and stops prematurely to create a vital capacity deficit.

These are the findings in a 42-year-old female in whom some parts of the lung were severely emphysematous and others less involved.

(table 1, *Case R.P.*, figure 7*A*). Measured response to bronchodilators is minor or even absent. Terminal volume is usually larger than shown in *Case R.P.*, amounting to more than a liter and exceeding one-fourth of the predicted vital capacity. The vital capacity deficit is large (*see* figure 4).

When the disease is not so advanced, useful volume is less restricted (*Case C.K.*, figure 7*A*). Flow is correspondingly faster but remains unresponsive to adrenalin. The size of useful volume and its prevailing rates of flow reflect inversely the clinical severity of the disease.

The outflow pattern for *Case R.P.* before bronchodilators resembles that of *Case J.H.* (*see* figure 5*B*) when the patient was seriously obstructed with secretions. Outflow for *Case C.K.* resembles that of *Case E.O.* (*see* figure 5*A*, an asthmatic). Identification of specific cause for the respective spirographic manifestations depends on the changes brought about by bronchodilators. Similarly, emphysema exhibits the most severe restriction of volume in the present series; but low $d\dot{V}/dV$ as well as large terminal volume serve to identify the obstructive nature of the condition. When disease which is primarily volume-restricting is complicated by emphysema (*see* table 1), tuberculosis for example, there is no way to assess the contribution of the lesion which directly limits bellows excursion. Indeed, its contribution cannot be detected even in the presence of concomitant obstructive disease. Dual causation limits specificity in the spirogram. Knowledge of any obstacle to bellows movement other than obstruction and knowledge of bronchodilator response are requisite if analysis is to be carried beyond the limited goals which the terms "restrictive" and "obstructive" imply.

Timed expiratory volumes, expressed as per cent of the total, are reduced in emphysema

chiefly by the large terminal volume. They may fail to reflect the response to bronchodilators. Through extension of terminal volume the vital capacity may be enlarged in proportion to the improvement of the volume in the first second. Thus, in *Case R.L.* (*see* figure 6*C*), the one-second expiratory capacity was 53 per cent of the vital capacity before bronchodilator and was 49 per cent afterward, despite an improvement in breathing.

Anatomic emphysema with bronchitis or asthma: The comparatively low value for $d\dot{V}/dV$ reflects the influence of greater bronchospasm before bronchodilators. The low value is improved afterward but is still depressed by the effect of emphysema. It is well to emphasize the great frequency with which asthma, bronchitis, and emphysema comprise complications of other conditions (*see* table 1). Combined disease makes the spirogram more difficult to interpret.

Knowledge of the complex conditions governing outflow in emphysema is incomplete, and the following explanation is offered with some reservation. It becomes explicable if the exceptional relation of tension to volume is taken into account. In the course of inflation, the advanced emphysematous lung, unlike the normal, remains slack till within a liter of full inspiration. Then tension mounts abruptly to a low level of pressure in the order of 10 cm. of water pressure or less (1, 8, 10, 11). Thereafter, tension increases little or not at all. Failure of tension to increase further led Christie (10) to conclude that its elastic limit had been exceeded. When the disease is less advanced, tension varies with volume more nearly, but maximal tension is low. These observations, reported earlier, were confirmed in 6 patients in the present series. It was further observed that, during expiration, tension reached zero at approximately the transition from useful to terminal volume. In all other lung conditions, critical flow parallels the decay of lung tension. It does so in emphysema with respect to the zero point. It might be expected to follow the course of lung tension earlier in the exhalation, with resulting low value for $d\dot{V}/dV$. *Case W. Os.* (figure 7*B*) is representative of advanced disease. *Case C. K.* (figure 7*A*) exemplifies less advanced disease. Maximal tension was only 14 cm. of water pressure. In linear relation to volume, it reached zero upon the exhalation of 2.3 liters.

Low flow rates are partly due to the small size

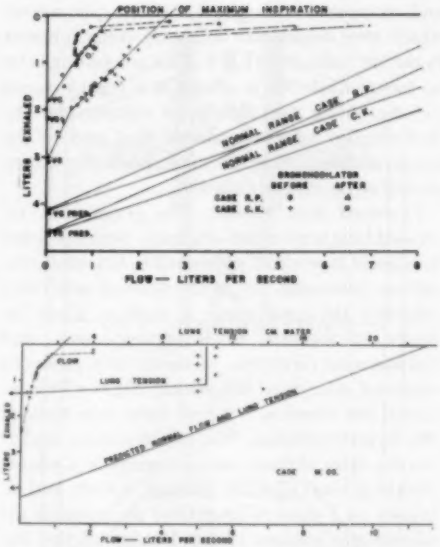

FIG. 7*A* (Upper). *Case R.P.* Severe, dry, anatomic emphysema, breathing capacity 34 L./min., little response to bronchodilators. The value for $d\dot{V}/dV$ remains low, 0.8, the numeral below the line tracing the course of critical flow. *Case C.K.* Moderate anatomic emphysema, breathing capacity 100 L./min., no response to bronchodilators. Lung tension was measured in this instance. Maximal lung tension was 15 cm. of water pressure. During exhalation it traced a linear relation to volume, reaching zero at the exhalation of 2.3 liters, approximately the place at which the transition from critical to terminal flow occurs. *B* (Lower). *Case W.Os.* Advanced, dry, emphysema; breathing capacity 33 liters per minute. By employing appropriate scales, lung tension and predicted normal flow can be plotted on the same line, in relation to volume. As measured, lung tension maintained a small plateau of pressure near the position of maximal inspiration. Exhalation beyond this range brought the tension abruptly to zero, approximately at the place at which transition from critical to terminal volume takes place.

of the compartment of relatively intact lung from which outflow of useful volume takes place and partly due to the fact that it is inflated to less than half the normal maximal tension. Thus, in *Case W.Os.*, with a useful volume of nearly a liter at half inflation, the compartment would comprise approximately half the parenchyma if it were normal structurally. Such an amount would give maximal critical flow of about 2.5 liters per sec-

ond, compared with the 1.25 liters per second which were found, the difference being inherent in the low value for $d\dot{V}/dV$. This accounting is by no means final, but is offered as a cogent reason for concluding that the useful volume and the flow entailed reflect the size of that part of the emphysematous lung remaining relatively intact, as well as its state of inflation.

Increased lung tension: The group with increased lung tension had originally been restricted to cases of interstitial pneumonitis, but other conditions possessing the same physical attributes exhibited the same course of outflow. These included scleroderma, lupus erythematosus, and lymphangitic carcinoma whenever they produced universal interstitial thickening. Signs which reflected the common physical state were tachypnea, hyperventilation, loud breath sounds, numerous fine rales, diffuse roentgenographic shadows, shrinking lungs over the passage of time, and alkalosis. In 6 cases of interstitial pneumonitis the microscopic sections revealed many of the features of Hammon-Rich disease.

In the spirogram, flow starts very rapidly, reaching 6 to 12 liters per second, and the effort-dependent phase is relatively large (*Case A.C.*, figure 8). Even the pressures of cough may not suffice to establish critical flow until volume is considerably depleted. The value for $d\dot{V}/dV$ is high (*see* table 1). Terminal volume is small and so is vital capacity (*see* figure 4). Breathing capacity is relatively good and may exceed the predicted normal. Note that the changes are opposite to those of emphysema.

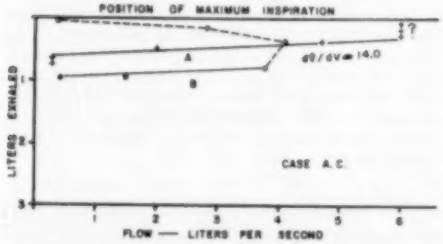

FIG. 8. *Case A.C.* Very advanced interstitial pneumonitis confirmed at necropsy. A: Successive coughs. Critical flow established only on the third cough and thereafter, giving very high value for $d\dot{V}/dV$. *B*: Maximal uninterrupted expiratory effort. Could not exert enough force to narrow airways and establish critical flow till near the end of expiration. Dotted line is effort-dependent phase.

Microscopic sections always show enormously dilated bronchioles, pulled wide by excessive tension of surrounding tissue. Lung compliance is low, not from the reduced amount of lung subjected to inflation, but from actual stiffening of parenchyma. Maximal lung tension may exceed 40 cm. of water pressure. This widens the bronchioles and accentuates the change of diameter for a given change of volume. Flow again conforms to tension, starting at high rates and decelerating rapidly with respect to volume. So far as it has been possible to discover, there is no disease characterized by loss of bronchiolar contractility which would produce the same dilatation as excessive external tension. Airflow manifestations of wide bronchioles are, therefore, diagnostic for this group of diseases. This is unique in spirometry. There are dual potential causes for all other alterations of flow and volume in the spirogram.

DISCUSSION

The factors governing expiratory flow are set forth in figure 9. A mechanical model based on this design can be made to demonstrate the action of these factors. Let the airways be represented by a single tube (AW) which is enclosed within a single space bounded by chest wall (CW) and diaphragm (D). Pressure at various points is indicated by the size of the stippled arrows (P). Pressure declines downstream, and the disparity of pressure with that on the outside compresses the tube in proportion to the amount of decline at a given point. This is the basic mechanical situation. Cartilagenous tension (CT) offers graded resistance to collapse, thereby regulating flow at a predetermined level under various expiratory efforts through the effect of effort on the canalicular gradient. Bronchiolar resistance likewise affects the gradient. This, in turn, depends on the equilibrium between the contractile force of bronchiolar tension (BT) and the dilating force of lung tension (LT). Since the latter varies with volume, the level of regulated flow, called critical flow, does likewise.

Bronchiolar spasm in asthma reduces rate of flow and change of flow with respect to change of volume, $d\dot{V}/dV$. In emphysema, low lung tension and low $d\dot{V}/dV$ create the same effect on flow as that prevailing in asthma except that the reduction in flow is unresponsive to bronchodilators. Interstitial pneumonitis is characterized by high

lung tension and low lung compliance, with resulting rapid flow and high value for $d\dot{V}/dV$, the opposite of emphysema. Two conditions impair cartilagenous resistance to compression. Wright (12) and Herzog (13) have demonstrated atrophy of bronchial cartilage in advanced emphysema. The same change has been observed in poliomyelitis with chronic respiratory paralysis. These seem to be secondary changes which would, nevertheless, aggravate the pre-existing condition.

When exhalation takes place from healthy lung through healthy airways, the value for $d\dot{V}/dV$ is remarkably constant, 2.7 ± 0.4. The amount of participating lung affects the volume exhaled but not the pattern of outflow defined by $d\dot{V}/dV$. This enhances diagnostic applicability of the above concept of mechanics. Diagnostic specificity is frequently impaired by the existence of dual potential causes for a spirographic feature. Foreknowledge of the action of bronchodilators and of lesions which limit the volume of displaceable air tends to dispel uncertainties.

Analysis proceeds as follows. If $d\dot{V}/dV$ is significantly elevated, 3.5 or more, useful volume and vital capacity will be small. Such findings signify generalized interstitial thickening. In such an instance, lung compliance varies inversely with $d\dot{V}/dV$.

If $d\dot{V}/dV$ is normal, 2.3 to 3.1, obstruction and interstitial thickening are excluded. If volumes are likewise normal, it can be stated that ventilation is normal. Reduced useful volume identifies one or another of the lesions which primarily limit volume. This includes voluntary restriction and the exclusion of part of the lungs from ventilation by secretions. Which of these factors is operative must be determined from the clinical record or, in the case of secretions, from the effect of a bronchodilator.

In practice, the majority of cases exhibit a low value for $d\dot{V}/dV$, less than 2.0, which specifies obstructive disease. If due entirely to bronchospasm, $d\dot{V}/dV$ can be restored to normal or nearly so by the action of a bronchodilator. Emphysema causes a fixed depression. In emphysema useful volume and the flow entailed probably reflect the amount of relatively intact parenchyma, as opposed to the emphysematous compartment. The findings suggest that it should be possible to derive other quantitative information. If $d\dot{V}/dV$ and compliance are known, lung tension can be computed from critical flow, since both are linked to volume. Capacity for flow at various levels of volume, $d\dot{V}/dV$, under a range of pressure, makes $d\dot{V}/dV$ inversely proportionate to the resistive state of the subject. A simple statement of resistance itself is not possible because of constant flow under various pressures.

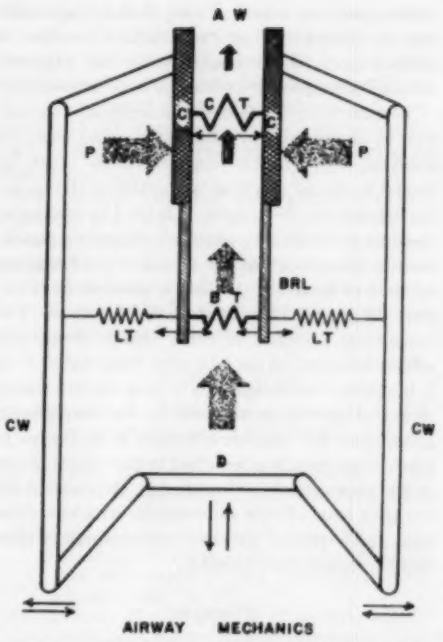

FIG. 9. Factors governing expiratory flow: *AW*—Airway; *C*—Cartilagenous portion; *BRL*—Bronchiolar portion; *CT*—Cartilagenous tension, illustrated by heavy spring which exerts a dilating force opposed to compression, indicated by arrows; *LT*— Lung tension, illustrated by delicate spring. Arrows indicate the dilating effect on bronchioles; *BT*—Bronchiolar tension exemplified by spring. The arrows indicate the narrowing effect on the bronchioles; *P*—Pressure in the terminal spaces, indicated by the size of the dotted arrows. Pressure declines downstream in the airways; *CW*—Chestwall. Arrows show direction of movement; *D*—Diaphragm. Arrows show direction of movement.

The spirogram serves its most useful purpose when it can disclose why breathing is abnormal. The breathing capacity test is an easy and adequate measure of the degree of abnormality which is not to be improved upon by substituting some quantitative aspect of the spirogram, nor is breathing capacity an acceptable standard for ap-

praising such an aspect. To say that a lung condition is "restrictive" or "obstructive" realizes a limited goal which might better be attained through a simpler procedure such as fluoroscopy.

To gain more fundamental insight into the nature of disease and to establish a sound basis for analysis, spirographic manifestations must be linked to causal physical properties of the lungs. An attempt has been made in table 1 to categorize diseases according to physical properties significant to the spirogram. By means of the foregoing method of analysis, it is often possible to designate the proper category from the spirogram. The concurrent presence of other disease frequently offers difficulty, as may be seen from table 1. It is hoped that investigations in progress will clarify doubtful points, particularly in the emphysema group, and will validate attempts to derive quantitative information described in this report. Even in the present state of knowledge, the method advocated here affords information which is more basic, more precise, and more discriminatory than has heretofore been possible.

Summary

In healthy subjects, the forced expiratory spirogram begins with a small effort-dependent portion. The second and largest part is characterized by orderly deceleration of flow at a rate of 2.7 liters per second for every liter exhaled. It ends when flow becomes 0.5 liters per second, a point at which lung tension has been found to reach zero. At any selected point in the second part, flow cannot be increased by applying greater than threshold pressure; hence, it is called critical flow. In the third terminal part, flow is slow and variable and volume is small.

Constant flow at a selected point is attributed to check-valve narrowing of cartilaginous large airways. The decelerating rate at which flow is regulated is ascribed to bronchiolar narrowing which parallels the decrease of volume and is mediated through decreasing lung tension. These characteristics are constant for all healthy lungs served by healthy airways. The amount of participating lung determines the inspiratory extremity of the spirogram, but the configuration is constant in health.

Disease produces quantitative variation in the characteristics exhibited in health. Bronchospasm makes bronchioles small and more resistant to stretch, so $d\dot{V}/dV$ is lower than the normal value of 2.7, but responds to bronchodilators. Interstitial pneumonitis renders bronchioles wide, so $d\dot{V}/dV$ is high. Secretions reduce the amount of participating lung and so do not alter $d\dot{V}/dV$. In emphysema, the fixed low value for $d\dot{V}/dV$ is ascribed to low lung tension and the aberrant relation to volume. In this disease, flow is judged to be sequential. First, a restricted volume is exhaled from relatively intact parenchyma. Thereafter, slow leakage from emphysematous parenchyma makes up an enlarged terminal stage of the spirogram. Value for $d\dot{V}/dV$ varies inversely with the flow-resistive state of the airway system.

A method of analysis based on the foregoing, and with ancillary knowledge of volume-restricting lesions and of the effect of bronchodilators, appreciably extends the useful information to be obtained from the spirogram. In an extensive clinical experience it has proved to be a helpful diagnostic measure.

Sumario

El Espirograma Espiratorio

En los sujetos sanos, el espirograma espiratorio forzado comienza con una pequeña porción debida al esfuerzo. La segunda y mayor porción se caracteriza por retardo ordenado de la corriente de aire a razón de 2.7 litros por segundo por cada litro exhalado, y termina cuando la corriente alcanza 0.5 litros por segundo, punto este en el que se ha observado que la tensión pulmonar llega a cero. En cualquier punto de la segunda porción escogido, no cabe aumentar la corriente aplicando una presión de más del triple; de ahí la designación de corriente crítica. En la tercera porción, la terminal, la corriente es lenta y variable y el volumen es pequeño.

La constante corriente en un punto escogido se atribuye a estrechamiento a modo de válvula de retención en las grandes vías aéreas cartilaginosas. La magnitud del retardo que se fija a la corriente se imputa al estrechamiento bronquiolar que refleja la disminución del volumen y se gradúa por la decreciente tensión pulmonar. Estas características son constantes para todos los pulmones sanos servidos por vías aéreas sanas. La proporción participante de pulmón determina la extremidad aspiratoria del espirograma, pero la configuración es constante en estado de salud.

La enfermedad produce una variación cuantitativa en las características mostradas en la salud. El broncospasmo reduce el tamaño de los bronquíolos y los hace más resistentes a la dilatación, de modo que $D\dot{V}/DV$ es menor del valor normal de 2.7, pero responde a los broncodilatadores. La neumonitis intersticial ensancha los bronquíolos de modo que $D\dot{V}/DV$ es alta. Las secreciones reducen

la proporción del pulmón que participa, de manera que no altera DV̇/DV. En el enfisema, el valor bajo fijado para DV̇/DV se imputa a la baja tensión pulmonar y la relación aberrante al volumen. En esta dolencia, se juzga que la corriente sigue cierto patrón. Primero, se exhala un volumen limitado por un parénquima relativamente intacto. A partir de ahí, el lento escurrimiento por el parénquima enfisematoso crea una etapa terminal agrandada del espirograma. El valor para DV̇/DV varía en razón inversa al estado de la resistencia a la corriente en el sistema de vías aéreas.

Un método de análisis basado en los datos anteriores, y con previo conocimiento de las lesiones que limitan el volumen y del efecto de los broncodilatadores, amplía apreciablemente la utilidad de la información obtenible del espirograma. En una extensa aplicación clínica, el mismo ya ha resultado ser una valiosa providencia de diagnóstico.

Resume

Le spirogramme expiratoire

Chez les sujets en bonne santé, le spirogramme en expiration forcée commence par une petite partie qui dépend de l'effort. La seconde et plus grande partie est caractérisée par la baisse régulière du débit à un taux de 2,7 litres par seconde par litre expiré. La fin se produit quand le débit atteint 0,5 litre par seconde, point auquel on a trouvé que la tension du poumon atteignait zéro. A n'importe quel point choisi dans la seconde partie, le débit ne peut pas être accru en appliquant une pression supérieure au seuil; par conséquent, on l'appelle le débit critique. Dans la troisième partie terminale, le débit est lent et variable, et le volume est petit.

On attribue un débit constant à un point choisi à un rétrécissement des larges voies aériennes cartilagineuses faisant soupapes. Le taux décroissant sur lequel le débit est réglé est imputé au rétrécissement bronchiolaire qui suit la baisse de volume et est obtenu par la diminution de la tension pulmonaire. Ces caractéristiques sont constantes chez tous les poumons sains desservis par des voies aériennes saines. La quantité de poumon en activité détermine l'extrémité inspiratoire du spirogramme, mais la configuration est constante en bonne santé.

La maladie produit une variation quantitative dans les caractéristiques montrées en bonne santé. Le bronchospasme rétrécit les bronchioles et les rend plus résistantes à l'étirement, ainsi dV/dV est inférieur à la valeur normale de 2,7, mais répond aux bronchodilatateurs. La pneumonie interstitielle élargit les bronchioles, dV/dV est ainsi élevé. Les secrétions réduisent la quantité de poumon en activité et ainsi ne change pas dV/dV. Dans l'emphysème, la valeur basse, fixe, de dV/dV est imputée à une tension pulmonaire basse et le rapport aberrant au volume. Dans cette maladie, le débit est, pense-t-on, en ordre de succession. D'abord un volume restreint est expiré par le parenchyme relativement intact. Puis, une fuite lente du parenchyme emphysèmateux contribue à un stade terminal élargi du spirogramme. La valeur de dV/dV varie inversement avec les conditions de résistance au débit du système des voies aériennes.

Une méthode d'analyse, basée sur les faits précédents et avec la connaissance auxilliaire des lésions diminuant le volume, et l'effet des bronchodilatateurs, élargit d'une façon appréciable l'information utile à obtenir du spirogramme. Au cours d'une expérience clinique étendue, ceci s'est montré un moyen de diagnostic utile.

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CORRELATION OF POSTMORTEM FUNCTION AND STRUCTURE IN NORMAL AND EMPHYSEMATOUS LUNGS[1, 2]

PHILIP C. PRATT, ANWARUL HAQUE, AND GEORGE A. KLUGH

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Introduction

In a previous publication (1) the writers have described techniques by means of which certain aspects of pulmonary function can be investigated in the lung excised post mortem. The studies accomplished include the determination of total lung volume, residual volume, inspiratory compliance, and expiratory flow rate. Methods were also described for the preparation of serial sections of dried inflated lungs for structural studies. It is the purpose of the present report to present details of the difference in structure and function between normal lungs and those with well-established clinically recognizable obstructive emphysema. Certain inferences are derived concerning the morphogenesis of this disease. Future papers will deal with the structural and functional details of lungs which show abnormalities of the character seen in emphysema but which have been obtained from persons without pulmonary complaints.

It should be emphasized that the functional tests used in this investigation are not intended to advance basic knowledge of the physics of pulmonary tissue. Rather they are used to provide objective knowledge of ventilatory and mechanical characteristics of lungs for correlation with structural studies carried out following fume fixation, drying in inflation, and serial sectioning. For example, upon finding structural defects in a lung obtained from a person who had had no pulmonary symptoms during life, the question immediately arises: Was this patient's ventilatory capacity really normal? On the other hand, when patent bronchioles are demonstrated to communicate with emphysematous foci in severely damaged lungs, as will be shown below, one wonders: Was this a true case of diffuse obstructive emphysema?

Since the above questions can only be answered by objective measurements and since pulmonary function studies during life are rarely available in connection with the autopsy material at this medical center, methods have been developed (1) for obtaining such measurements, using the excised lung. The tests used are comparatively simple, and it is recognized that other workers (2–4) have reported considerably more precise and elaborate measurements concerning the mechanics and physics of excised lungs.

Materials and Methods

The lungs included in this study have all been obtained from the general autopsy service of the Ohio State University College of Medicine. Clinical case histories have been reviewed in each instance, and the lungs have been separated into two groups on the basis of the presence or absence of respiratory complaints, past history, and physical findings of a character compatible with obstructive emphysema. Cases of prolonged history of progressive respiratory difficulty and the gross appearance of diffuse and bullous emphysema were placed in the emphysematous group and most had been so diagnosed by clinicians, although a few patients who were first seen during their terminal illnesses were thought to have primary cardiac disease. Unfortunately, none of the persons from whom lungs have been obtained during the eighteen months in which this investigation has been in progress have had studies of pulmonary function carried out during life. The writers are still awaiting an opportunity to observe the correlation between postmortem functional activity of the lungs and the measurements obtained during life.

The methods used for postmortem functional studies have been described in detail previously (1) and will not be repeated here. Suffice it to say that the residual volume as determined in this investigation is the volume of air remaining in a lung after ten minutes of free collapse from the fully inflated state. The total volume is the sum of the residual volume plus the total amount of air which the lung can be made to aspirate when exposed in a respirator box to negative pressure of a degree determined to be proper for each individual lung, usually between 10 and 15 cm. of water. The inspiratory compliance curves are obtained by recording separately the changes in pressure within the respirator box and the changing volume of the lung during a dynamic inflation of the lung; the pressure is then plotted against the volume.

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[2] This study was supported in part by research grants from the Ohio Thoracic Society.

The expiratory flow rate curve is simply the spirometer tracing of the deflation of the lung from full inflation following sudden release of the "negative pressure."

To date the writers have had the opportunity to study seven lungs from persons with the clinical picture of diffuse obstructive emphysema. The age range of these patients was from fifty-two to eighty years, and all were males. The known duration of symptoms was from five to twelve years. Causes of death were respiratory failure, 4; cor pulmonale with congestive failure, 2; and hemorrhage from gastric ulcer, one. All cases had morphologic cor pulmonale.

There were 21 lungs from persons without pulmonary complaints which are therefore classified as normal for present purposes. The age range was from twenty-three to seventy-six, and 13 patients were males. Many different diseases were identified as causes of death in these cases, but none were fundamentally pulmonary.

Results

A. Functional Studies

As noted in the previous publication (1), all lungs were subjected to a "static inflation" before the dynamic studies were conducted. Curves obtained with a normal lung and an emphysematous lung during static inflation are shown in figure 1. Methods are explained in the legend of figure 1. It may be noted that the emphysematous lung inflated readily at pressures much lower than those required for the normal lung and that the emphysematous lung reached its maximal size at a pressure of 10 cm. of water, while 12 cm. were required to fully inflate the "normal" lung. This is a characteristic difference between normal and emphysematous lungs as shown in table 1. It should also be noted that the maximal pressure established in this wa · was not exceeded by more than 2 cm. of water in subsequent tests because of the risk of producing structural artefacts.

Typical dynamic inspiratory volume curves for the normal and emphysematous lungs represented in figure 1 are shown in figure 2. All other lung studies gave similar curves. The rate of inspiration is essentially the same for each, 250 cc. per second, because this was the standard rate at which the air was being removed from the box. The volume of air inspired by the emphysematous lung was greater than that for the normal one. The records obtained for pressure changes in the box occurring simultaneously with the volume changes represented in figure 2 are demonstrated in figure 3. For the normal lung the pressure increased steadily as the volume increased while, in the case of the emphysematous lung, the pressure first increased, then dropped back, and finally increased again to its maximum. This phenomenon has been present consistently in the seven lungs from persons with clinical emphysema.

In figure 4 the volume changes recorded in figure 2*A* and *B*, and the pressure changes recorded in figure 3*A* and *B* have been plotted against each other by determining from the charts the volume and pressure at identical time intervals after onset of inflation. The curves in figure 4, since they show the relationship between volume change and pressure change, actually represent the changing compliance of the lungs during inspiration. The drop in pressure during inflation of the emphysematous lung seen in figure 3*B* is reflected in the compliance curve as an S-shaped bend, suggesting that the lung is supercompliant at this stage of inflation.

Figure 5 is a composite graph showing a comparison of all findings as to functional activity of the normal and emphysematous lungs studied to date. For clarity, only the largest and smallest of the normal and emphysematous lungs have been plotted. The total volume of all remaining lungs fell between those plotted, and the curve for the smallest emphysematous lung was lower than that for any other at all pressures. The compliance curves for a few of the normal lungs were higher than the one plotted, and several overlapped the emphysema curves in the pressure range from 4 to 10 cm. Each plot begins at the left at the level of the residual volume of the individual lung. Each curve extending from this point represents the dynamic inspiratory compliance of the lung. Evidence of "supercompliance" was obtained with every emphysematous lung. Note again that the emphysematous lungs reach their maximal volumes at lower pressure levels than those required for normal lungs. The highest point plotted for each lung represents its total volume, and this is consistently higher for emphysematous lungs than for normal lungs. Expiratory flow rates are plotted at the right. Note the difference in contour of the curves obtained with emphysematous as compared with normal lungs.

In table 2 individual data are presented for each lung examined, for the residual volume as determined by water displacement, the total volume, and the predicted total volume calcu-

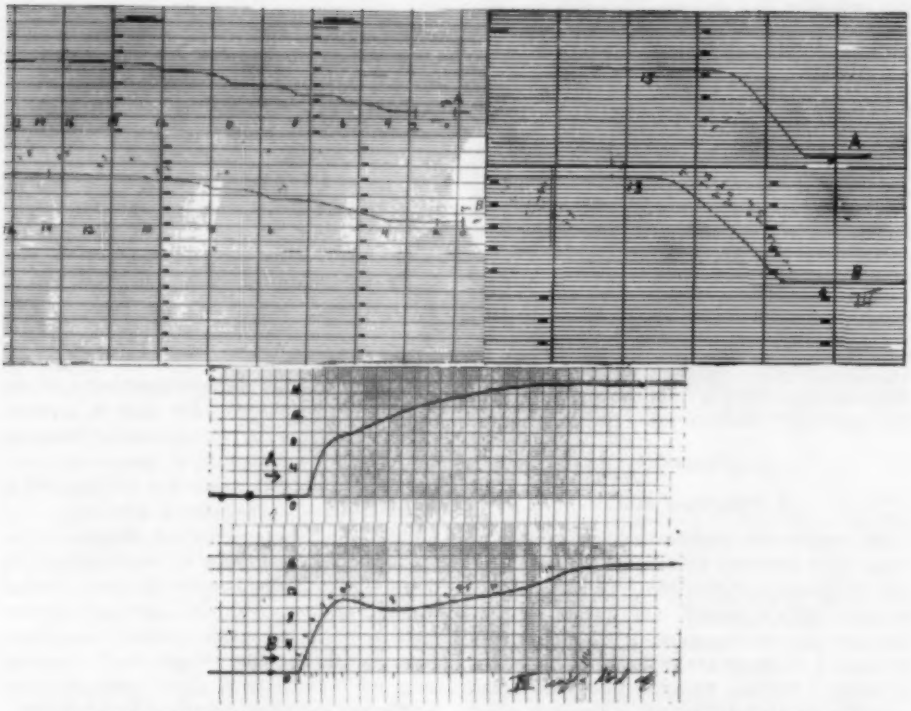

FIG. 1 (Upper left). Typical static inflation curves obtained with a normal lung (*A*) and one with obstructive emphysema (*B*). The tests were done separately. The lung was suspended in a respirator box with the bronchus connected to a spirometer. Suction was then applied to the box so as to diminish the pressure within it in steps of 2 cm. each. The tracings extending from right to left from points *A* and *B* show the volume changes in the lungs occurring in response to each pressure change. Note that the largest volume change for the normal lung occurred between 8 and 12 cm. and, for the emphysematous one, between 4 and 8 cm. Note also that for the normal lung no appreciable volume change occurred when the pressure was changed from 12 to 14 or 16 cm. In the emphysematous lung, the volume changed but little when pressure changed from 8 to 10 cm., and there was no change from 10 to 12 or 14 cm. In other words, the normal lung became fully inflated at 12 cm., and the emphysematous one at 10 cm.

FIG. 2 (Upper right). Dynamic inflation curves for normal (*A*) and emphysematous (*B*) lungs. The tracings again extend from right to left. The slope of the lines represents the rate of air flow which was about 250 cc. per second in each instance. Note that the emphysematous lung inspired more air than the normal one and that for each lung the rate of inspiration was essentially constant.

FIG. 3 (Lower). Pressure recordings obtained simultaneously with the volume curves shown in figure 2. These records were obtained by means of a strain gauge and electronic recording apparatus. While there are variations in the rate of pressure change during inflation of the normal lung (*A*), the pressure does continuously increase to its maximum. In the case of the emphysematous lung (*B*), pressure first increases, then drops back slightly, and finally increases to the maximum.

lated from the height and age of each patient according to the formula presented by Kaltreider and associates (5). It should be noted that, while the total volume of some normal lungs was several hundred cubic centimeters above the prediction, others were lower, and the average observed volume was within 150 cc. of the prediction. All of the emphysematous lungs were

more than 1 liter larger than predicted, and the average total volume was some 1,500 cc. above the prediction.

B. Structural Studies

The most informative areas in regard to evaluation of pulmonary structure are those in which bronchioles and related alveolar ducts and alveoli have been cut longitudinally. In such areas correlations may be observed between alveolar alterations and those in alveolar ducts and bronchioles. In figures 6 and 7, the characteristic relationship of these structures is shown in lungs from persons without pulmonary complaints and with "normal" postmortem function studies. The size of bronchioles, alveolar ducts, and alveoli are noteworthy, as well as the intact character of the membranes composing them. Early emphysematous changes observed in a lung from a person without pulmonary complaints and with normal postmortem functional data may be seen in figure 8. The relationship of bronchioles and alveolar ducts to emphysematous areas in lungs from patients with clinical emphysema and abnormal postmortem function studies are set forth in figures 9, 10, and 11. It is apparent that the bronchioles were but little changed from normal and that the major abnormalities were in the alveolar septa. The prominent abnormality was the presence of perforations in the alveolar septa. These perforations were of various sizes, and the large ones clearly represented a complete loss of one or more alveolar septa. It may be seen that widely patent bronchioles and alveolar ducts opened directly into the bullae formed by loss of septal tissue. These structures were patent despite the demonstrated presence of air trapping during expiration.

TABLE 1

PRESSURES REQUIRED FOR MAXIMAL INFLATION OF NORMAL AND EMPHYSEMATOUS LUNGS

Classification	Number	Pressures in Cm. of Water	
		Range	Average
Normal	21	10 to 14	12
Emphysema	7	8 to 10	9

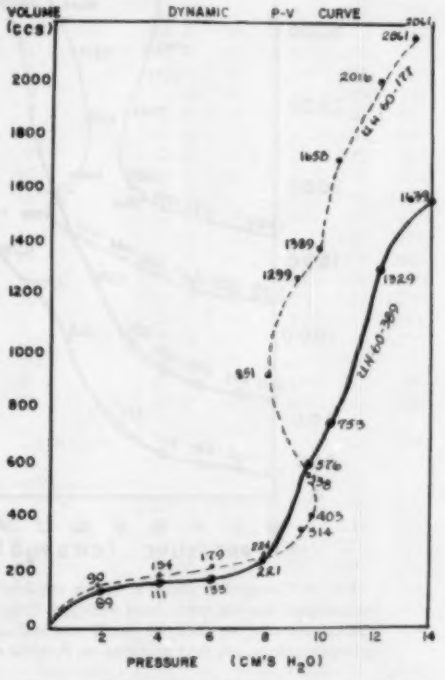

Fig. 4. Dynamic inspiratory pressure-volume curves for a normal lung (U 60-389) and an emphysematous one (U 60-177). These curves were prepared by determining at specific time intervals after onset of inflation (from the records shown in figures 2 and 3) the pressure in the box and the volume of the lung. These pressures and volumes were plotted against each other. The drop in pressure seen in figure 3*B* appears here as the "S"-shaped bend in the curve for lung No. 177.

DISCUSSION

The data presented above show that excised lungs from persons who died of advanced obstructive emphysema manifested mechanical findings identical in character to those observed during life. It is already well known that the abnormalities in clinical pulmonary function are readily demonstrable in advanced cases of obstructive pulmonary emphysema. The purpose of the present paper is to demonstrate the classic findings as a baseline for comparison in cases of early obstructive emphysema or in diseases which appear similar but may be fundamentally different. The types of functional and structural changes observed in such cases will be the subject of future publications.

Recent writers have presented various ana-

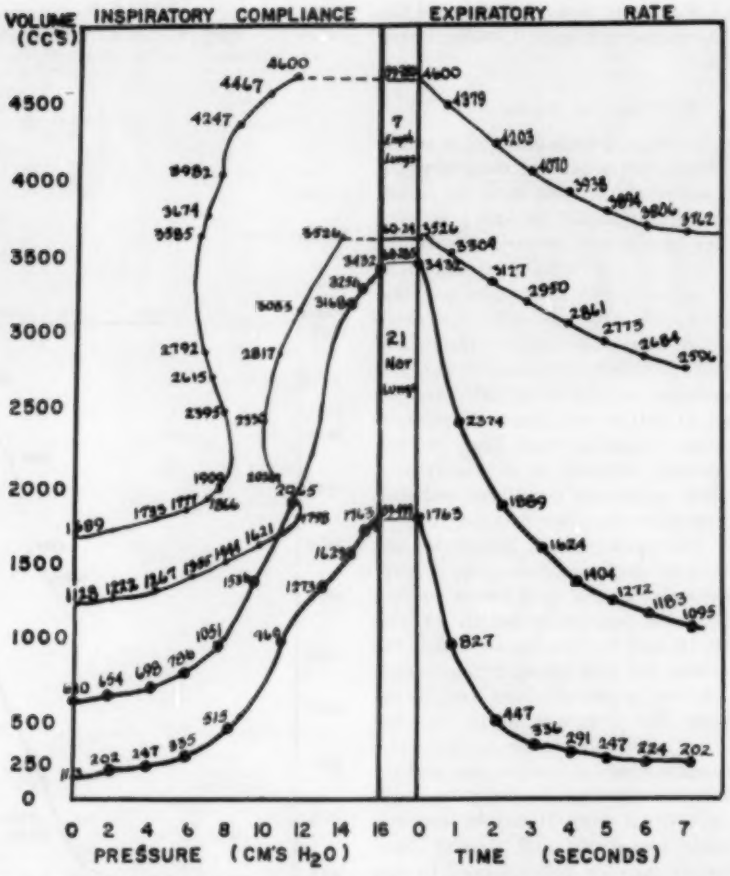

FIG. 5. Composite chart showing residual volumes (points of origin at left), inspiratory compliance curves, total lung volumes (highest point), and expiratory flow rates (right) for the largest and smallest of the 21 normal and 7 emphysematous lungs studied to date. For interpretations, *see* text sections on Results and Discussion.

tomic classifications of pulmonary emphysema based on the distribution of lesions. Types described include panlobular, centrilobular, and irregular emphysema (6). It is not the purpose of the present paper to identify the relationships or distinctions between these various types of pulmonary involvement. However, it should be mentioned that at least one example of each form is included among the 7 cases of obstructive emphysema and that it has been possible to demonstrate patent bronchioles and alveolar ducts communicating with lesions in all cases.

With regard to the specific functional phenomena demonstrated in the excised lungs, the fact that the lungs of persons with obstructive emphysema are excessively compliant has long been known (7, 8). The evidence of "supercompliance" shown during inflation of the excised emphysematous lungs, that is, the increasing volume while pressure decreases, was unexpected and has not been demonstrated during life. This phenomenon is believed to be in essence an artefact resulting from the methods employed for postmortem functional studies. It appears that during deflation of the excised lungs without chest wall limitation of minimum size, the lung

collapses to the point where surfaces of bronchioles or perhaps alveolar ducts actually come into contact. Fluid surface forces then tend to hold these together. During inflation a certain pressure is necessary to separate these surfaces, and little or no air can flow until they are separated. Once separated, the lung tissue itself is so slack that the lung readily inflates at a rate exceeding that at which air is being sucked out of the box; therefore, the pressure drops.

One may question why the same phenomenon does not occur in excised normal lungs. It seems necessary to assume that surfaces do become apposed during deflation of normal lungs without chest wall limitation. The difference appears to be that the tissue resistance to inflation is higher than that of emphysematous lungs and that this resistance, even in the deflated lung, requires for inflation a pressure above that necessary to separate the surfaces. The initial low-slope phase of the compliance curve of normal lungs is probably a manifestation of the force required to separate such surfaces.

"Tissue resistance" is a rather poorly understood phenomenon to which the present writers have nothing to contribute at this time. This has been studied extensively by others (3, 4), and it is clear that the major factor responsible for it is the surface tension of the fluid which lines the pulmonary membranes. However, the collagen and elastic tissue fibers of the pulmonary stroma do play a part. In emphysema there is known to be a decrease in surface area within the lungs, and there are abnormalities in structure and distribution if not in the character of the collagen and elastic tissue. The relationship of these factors, or the existence of others, in regard to supercompliance of emphysematous lungs remains to be determined.

The curves plotted in figure 5 all begin at the level of the residual air volume as determined by water displacement. The fact that excised emphysematous lungs have an increased residual volume as compared with excised normal lungs is evident. The close correlation of the average total volume of the 21 normal lungs, 2,242 cc., with the predicted value based on age, sex, and height, 2,113 cc., is worthy of note (table 2). It suggests that the procedure used for ascertaining the proper amount of pressure to be applied to each lung is valid. On the other hand, the marked excess of the average total volume determined for emphysematous lungs, 3,929 cc., over the prediction, 2,425 cc., confirms the impression that in emphysema not only is the residual volume increased, but also the total lung capacity. This confirms the plethysmographic observations obtained during life (9).

TABLE 2

INDIVIDUAL DATA FOR RESIDUAL VOLUME, TOTAL VOLUME, AND PREDICTED TOTAL VOLUME OF 21 NORMAL AND 7 EMPHYSEMATOUS LUNGS

Case Number and Classification	Sex and Age	Height	Total Lung Volume (One lung, right or left)		Residual Volume
			Predicted	Observed	
		inches			
Normal					
1	59 F	57	1,755	1,763	113
2	41 M	72	2,460	1,969	356
3	65 M	68	2,785	2,110	245
4	29 F	59	1,600	1,170	510
5	43 M	65	2,190	1,825	650
6	52 M	70	2,500	3,085	721
7	65 F	62	1,560	2,326	355
8	65 M	69	2,150	1,739	560
9	58 F	63	1,710	2,322	450
10	23 F	66	1,815	867	305
11	65 M	68	2,280	2,553	355
12	75 F	61	1,750	2,125	679
13	63 M	68½	2,325	2,626	721
14	46 M	65	2,165	3,001	609
15	40 M	71	2,460	3,432	610
16	38 M	68	2,395	2,969	577
17	73 F	62	1,775	2,340	1,188
18	40 M	65	2,260	2,416	733
19	76 F	50	1,165	1,987	870
20	53 M	67	2,410	2,735	633
21	57 M	66½	2,860	1,719	390
Averages			2,113	2,242	554
Emphysema					
1	68 M	69½	2,305	3,608	1,261
2	73 M	68½	2,680	4,600	1,689
3	67 M	65	2,660	3,524	1,963
4	63 M	65	2,210	3,526	1,178
5	65 M	69	2,325	3,996	2,200
6	66 M	66	2,210	3,704	1,643
7	80 M	68	2,585	4,455	1,913
Averages			2,425	3,929	1,692

The flat slope of the plots of spontaneous expiratory flow rates in emphysematous lungs, at the right in figure 5, is a manifestation of obstruction to air flow or "trapping" during expiration. The curves cover only the first seven seconds of the expiration. The contrast with the slopes of the normal lungs in the first seconds

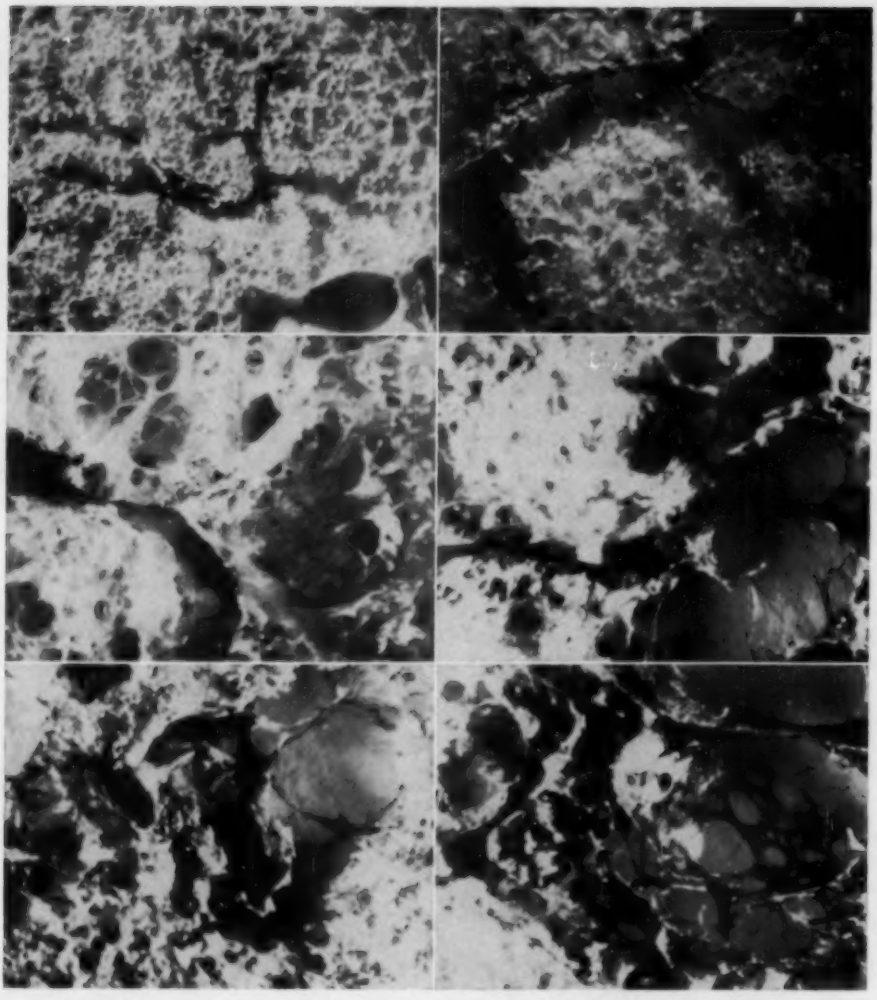

FIG. 6 (Upper left). Normal lung of 23-year-old patient. This field demonstrates the pattern of distribution of alveolar ducts. A small bronchus with associated pulmonary artery is seen in cross section in the right lower corner. This branches toward the left, and the branch immediately divides into two, one extending down and the other toward the center of the field with a short segment above the level of the section. This bronchiole then branches to left and right and becomes a respiratory bronchiole. Further branches are alveolar ducts. Note distribution of these latter, especially the one in the right lower quadrant which supplies air to the alveoli adjacent to the bronchus of origin. (7×) (reduced ½)

FIG. 7 (Upper right). Normal lung of 65-year-old patient. A terminal bronchiole enters the field and branches at the left, becoming a respiratory bronchiole. Subsequent branches at right are alveolar ducts. This higher magnification is intended to reveal the intact character of the membranes composing these structures and also the alveolar septa. Pores of Kohn are too small to be visible. (20×) (reduced ½)

FIG. 8 (Center left). Early emphysema in 65-year-old patient. The postmortem functional activities of this lung were normal as was the structure in most areas. In this field, however,

is striking. It might be suggested that the curve is flatter for the emphysematous lungs because less force is available to collapse them since they are inflated by only 8 to 10 cm. of water. However, if normal lungs are inflated at this pressure and allowed to collapse, the spirometer tracing still begins with a steep slope.

It may be noted also that, after sudden release of the inflating force, the normal lungs reach virtually the same volume as the residual volume within the first three or four seconds. In the emphysematous lungs the difference between the volume at the end of seven seconds and the residual volume indicated at the left is the amount of air which leaves the lung during a ten-minute period of free collapse.

In view of the impressive evidence of obstruction to air flow during collapse of emphysematous lungs, it is of considerable interest and importance to note that patent bronchioles and alveolar ducts are readily demonstrable in sections of these lungs prepared after inflation and drying. These structures are patent even in sites where they communicate with emphysematous foci (figures 9–11). Numerous similar examples have been found in each of the 7 emphysematous lungs studied to date. They are more easily demonstrable in small early lesions than in massive bullae because the entire periphery of a small lesion can be observed in a few serial sections, while often a dozen or more sections must be examined in large lesions. Since obstruction to air flow has been demonstrated to be present in these specific lungs, it seems necessary to explain it on the basis of collapse of the walls of the bronchioles or alveolar ducts during exhalation. Such collapse presumably occurs because of the loss of tissue tension which in the normal lung tends to hold them open. This mechanism has been suggested as contributing to air-flow obstruction in advanced emphysema by various writers (10, 11).

The observation that patent bronchioles communicate with emphysematous lesions has a significant bearing on concepts of the pathogenesis of pulmonary emphysema. Many writers, particularly McLean (12), have stated that obliteration of bronchioles by fibrosis or inflammation is the first step in the development of obstructive emphysema and that the trapping of air and the mechanical effects of breathing and coughing cause destruction of alveolar septal tissue. The findings shown here, especially the ease with which communications can be demonstrated between bronchioles and early emphysematous foci, are considered to be incompatible with that concept. Instead, the evidence indicates that the primary abnormality in pulmonary emphysema occurs in the alveolar septa and alveolar ducts and that obstruction to air flow, even in the earliest stages, is the result rather than the cause of the disease.

The septal abnormality manifests itself in the form of excessive numbers and sizes of fenestrations in the alveolar and alveolar ductal membranes. Several recent papers have drawn attention to these lesions (13, 14), as have many previous ones even as early as 1848 (15, 16). As these fenestrations enlarge, they gradually cause complete loss of many alveoli, their original volume being occupied by coarse spaces which often are crossed by residual strands of tissue.

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there are many examples of abnormal fenestrations in the membranes, best seen in the left upper quadrant. Many alveolar spaces have coalesced to form small bullae. The related bronchiole appears normal. (20×) (reduced ½)

FIG. 9 (Center right). Severe obstructive emphysema in 63-year-old patient. A respiratory bronchiole enters the field at the lower left, and promptly changes to an alveolar duct. This duct opens widely into the emphysematous bulla at the right, without evidence of structural constriction. This bronchus has been traced proximally to the hilum of the lung and is patent, as are those in the other illustrations. (20×) (reduced ½)

FIG. 10 (Lower left). Severe obstructive emphysema in 63-year-old patient. A terminal bronchiole is seen in cross section at left center. It leads to a tortuous respiratory bronchiole and alveolar duct which opens widely into the bulla at the right. The mechanism by which this alveolar duct might be mechanically collapsed and occluded during expiration is obvious. (20×) (reduced ½)

FIG. 11 (Lower right). Severe obstructive emphysema in 68-year-old patient. A respiratory bronchiole enters the field at the left and branches into two alveolar ducts, each of which communicates widely with the bullous area at the right. Note fenestrations developing in the membrane composing the wall of the bulla. (20×) (reduced ½)

The fundamental mechanism responsible for the development of the fenestrations remains to be identified.

Summary

Studies of the postmortem mechanical and ventilatory characteristics of normal and emphysematous lungs reveal that the latter are excessively compliant, have increased total volume and residual volume, and manifest obstruction to air flow during exhalation. Structural studies of the dried inflated emphysematous lungs reveal patent bronchioles communicating with both early and advanced emphysematous areas.

These findings are considered to refute the theory that bronchiolar obliteration causes the development of emphysema and to indicate that the primary abnormality in pulmonary emphysema occurs in the septal membranes of alveoli and alveolar ducts. Obstruction to air flow is considered to be the result rather than the cause of diffuse pulmonary emphysema.

Sumario

Correlación Después de la Muerte de la Función y la Estructura en los Pulmones Normales y los Enfisematosos

Estudios de las características mecánicas y ventiladoras de los pulmones normales y enfisematosos después de la muerte revelan que los últimos son excesivamente dóciles, muestran aumento del volumen total y del volumen residual y manifiestan oclusión del paso del aire durante la exhalación. Los estudios estructurales de los pulmones enfisematosos inflados y desecados revelan bronquíolos permeables que se comunican con las zonas enfisematosas tanto incipientes como avanzadas.

Se considera que estos hallazgos refutan la teoría de que la obliteración bronquiolar ocasiona la formación del enfisema e indican que, en el enfisema pulmonar, la anomalía primaria sobreviene en las membranas de los tabiques de los alvéolos y los conductos alveolares. Se considera que la oclusión del paso del aire es el resultado más bien que la causa del enfisema pulmonar difuso.

Resume

Corrélation entre la fonction post-mortem et la structure dans des poumons normaux et des poumons emphysémateux

Des études des caractéristiques mécaniques et ventilatoires post-mortem de poumons normaux et de poumons emphysémateux révèlent que ces derniers sont excessivement souples, ont un volume résiduel, et un volume total accrus, et montrent une obstruction au débit de l'air pendant l'expiration. Des études de structure des poumons secs, gonflés, montrent des bronchioles ouvertes communicant, à la fois, avec des régions emphysémateuses à un stade précoce et à un stade avancé.

Ces données réfutent, considère-t-on, la théorie qui prétend que l'obstruction bronchiolaire est la cause du développement de l'emphysème, et indiquent que l'anormalité primaire, dans l'emphysème pulmonaire, se produit dans les membranes septales de alvéoles et dans les canaux alvéolaires. L'obstruction au débit d'air est considérée comme le résultat plutôt que la cause de l'emphysème pulmonaire diffus.

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THE HEAT RESISTANCE OF TUBERCLE BACILLI IN THE LUNGS OF INFECTED MICE[1]

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Introduction

Schultz and Ritz (1) found that heating at 53°C. for twenty-five minutes caused complete killing of a four-hour culture of coliform bacilli, whereas the same heat treatment caused no reduction in the viable count of seven- to thirteen-hour cultures. Winslow and Walker (2) quote further evidence that heat resistance is related to the metabolic activity and speed of cell division during the aging of a bacterial culture.

In chronic tuberculous infection in the mouse, the viable count of culturable bacilli in the lungs may remain constant for long periods, and the lesions show no change or only slight progression (3–6). The constant level of bacillary counts may be explained either as a static persistence of nonmultiplying organisms, or as a dynamic equilibrium between multiplication and destruction. Rees and D'Arcy Hart (7) compared culturable and total bacillary counts of bacilli in the lungs of such mice and found little difference between these counts. This indicated that the majority of the bacilli in the lungs were viable, and they interpret this as evidence in favor of the static hypothesis. This conclusion was subject to the proviso that dead organisms were neither being removed from the lungs nor losing their acid-fastness. They investigated this possibility by treating infected mice with a combination of pyrazinamide and isoniazid; in these treated mice the culturable counts fell rapidly, while the total counts remained steady for three weeks, and then fell only gradually. This indicated that, following chemotherapy, a high proportion of dead organisms remain in the lungs and retain their acid-fastness, contributing to the total counts.

The purpose of the present work was to test Rees and D'Arcy Hart's hypothesis of a static bacillary population, using heat resistance as a criterion of the state of the bacilli. It was first necessary to show that multiplying and nonmultiplying tubercle bacilli differed in their resistance to heat. The technique was then applied to investigate the bacilli in the lungs of chronically infected mice; these mice were among those employed in the work of Rees and D'Arcy Hart (7). In addition, mice with acute progressive tuberculosis were included in the experiments; in such animals a proportion of the bacilli should be multiplying, and it was hoped that the comparison of the two groups of mice would add further weight to any conclusion reached.

Material and Methods

Mycobacterium tuberculosis strain H37Rv, or its streptomycin-resistant variant, H37RvSR, was grown in 7H-10 standard transfer medium (8) modified by the omission of glycerol. Samples of about 2 ml. in 6 × ⅝-inch test tubes were exposed in a thermostatically controlled water bath to a temperature of 53°C. (±0.25°C.), unless otherwise stated.

Counts of the culturable organisms were carried out in 7H-10 medium solidified by silica gel (9). The composition of the medium was such that, after the addition of silica sol, the concentrations of the constituents were the same as in the 7H-10 standard transfer medium, with certain modifications: albumin was replaced by oleic acid–albumin complex (8); Tween® 80 was omitted, and replaced by glycerol to a final concentration of 0.2 per cent; the concentration of ferrous ammonium citrate was increased to 0.08 per cent to allow for the absorption of ferrous ions (9).

Infected female albino P-strain mice (initial weight 18 to 20 gm.) were provided by Drs. R. J. W. Rees and P. D'Arcy Hart. The infecting organism was H37Rv, grown for two to three weeks as a surface growth in Proskauer and Beck medium. The pellicle was ground and suspended in 0.01 M phosphate buffer (pH 7.0) to the required opacity. To produce an acute infection ("acute mice"), 0.1 mg. wet weight of bacilli in 0.5 ml. was inoculated intravenously. The dose to produce a chronic infection ("chronic mice") was 0.02 mg.

The "acute" mice were sacrificed on the fifteenth day after inoculation, and the "chronic" mice at about four months. The lungs were removed aseptically, ground, and suspended in 10 ml. of phos-

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phate buffer. Samples for heating were transferred to the water bath with a minimum of delay.

In one experiment, the effect of diseased lung on the heat resistance of old and young cultures was investigated. Cultures of H37RvSR were diluted 1 in 10 in lung suspensions from both "acute" and "chronic" mice, and exposed to heat. Culturable counts were done in medium containing 100γ of streptomycin per ml.; this prevented the growth of the drug-susceptible infecting bacilli, but had no inhibitory effect on the drug-resistant bacilli which had been added.

Results

Several experiments were carried out to determine which temperature would most rapidly kill young, actively growing bacilli while having little effect on old, stationary phase bacilli. The results of one of these experiments are shown in figure 1.

It may be seen that exposure to 53°C. for forty-five minutes caused a thousand-fold fall in the culturable count of a young culture, but only a fourfold drop in the count of an old culture. The killing rate at 50°C. was too slow to be of practical value in differentiating between young and old cultures while, at 55°C., there appeared to be too-rapid killing of the old culture (confirmed in other experiments in which exposure to 55°C. was continued for longer periods). In all subsequent experiments a temperature of 53°C. was employed.

The effect of exposure to 53°C. over a period of two hours is shown in figure 2. The $\log_{10}$ of the count of culturable bacilli from a young culture fell from 6.96 to 0.40, whereas the count of an old culture fell from 8.62 to 7.28 over the same period.

A series of heat-resistance curves obtained at different stages in the growth of a culture is shown in figure 3. In this experiment a seven-day culture of H37RvSR was inoculated heavily into fresh medium. A sample was immediately removed and tested for heat resistance (day 0); the remainder was incubated at 37°C., and samples were removed at intervals for counts of culturable bacilli and for heat-resistance tests. On day 3, the culture was in the logarithmic phase of growth and there was rapid killing on exposure to heat, the culturable counts showing a fall of 6.2 log units over the two-hour period. By day 6, the culture was almost fully grown and

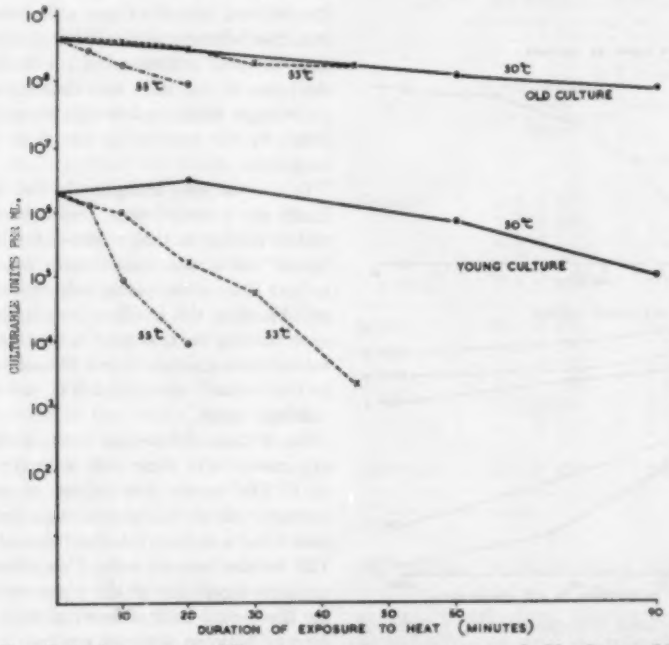

FIG. 1. The effect of exposure to different temperatures on young and old cultures of H37Rv.

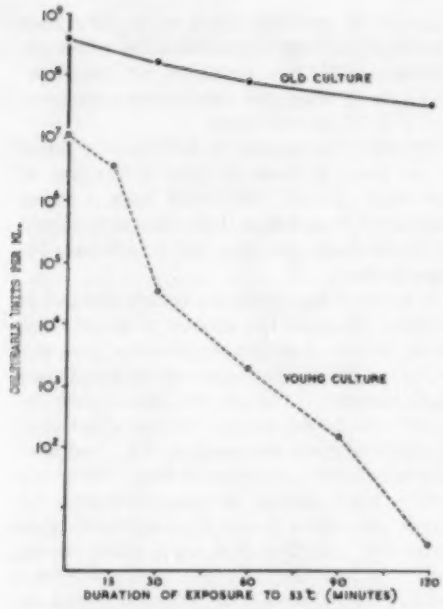

FIG. 2. The effect of exposure to 53°C. on young and old cultures of H37Rv.

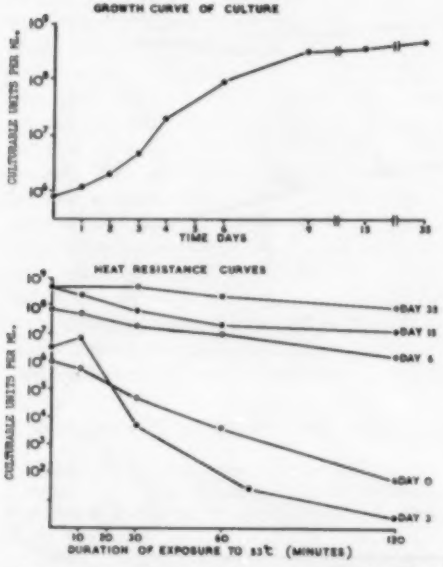

FIG. 3. The heat resistance of a culture of H37Rv at different stages in its growth cycle.

the heat-resistance curve was much flatter, the fall in count being 1.8 log units. The heat resistance on days 15 and 35 was similar to that of day 6, with a slight tendency toward progressively increasing resistance; the falls in count were 1.5 log units on day 15 and 0.9 on day 35. The source culture contained 5×10^7 viable units per ml., so that the heat-resistance curve at day 0 is presumably that of a culture in the late logarithmic phase; it falls intermediate between the curves at days 3 and 6, with a fall in count of 4.2 log units.

Heat Resistance of Bacilli in the Lungs of Mice

In the experiment illustrated in figure 4, two "acute" and two "chronic" mice were sacrificed. Heat-resistance tests were performed on the infecting bacilli and on old and young cultures of H37RvSR added to the lung homogenates.

The results with the old and young cultures were clearly distinguishable, showing that the presence of lung tissue did not mask the difference in heat resistance found in the preliminary experiments. In addition, there was no evidence that the "acute" and the "chronic" lung tissue differed in their effect on the heat resistance of the cultures added to them. It was concluded from the behavior of the old and young cultures that the heat resistance of the bacilli infecting the lungs of the mice was determined by their physiologic state, and was not significantly affected by the presence or nature of the diseased lung tissue.

It may be seen in figure 4 that the infecting bacilli in "chronic" mice had a heat resistance closely similar to that of the old culture. In the "acute" mice, the bacilli were less susceptible to heat than in the young culture, but more susceptible than the bacilli infecting the "chronic" mice: during the first hour of heating, the falls in culturable organisms were 1.78 and 1.06 log units in the "acute" mice, and 0.43 and 0.61 in the "chronic" mice.

Since these differences were small, a further experiment was done with a larger number of mice. The results are shown in table 1. The average fall in culturable organisms over the first hour was 0.45 for the "chronic" mice and 1.27 for the "acute" mice. This difference is statistically significant at the 1 per cent level. During the second hour of heating there was no difference between the two groups.

Discussion

It is clear that, in heat-resistance tests, bacilli in the lungs of mice with chronic infections behave like old rather than young cultures. The possibility that actively growing bacilli might appear resistant to heat owing to the presence of lung tissue was investigated in the experiment in which cultures were added to lung suspension and exposed to heat. The results showed that, although the young culture appeared to receive some degree of protection from the lung tissue present, it was still considerably more susceptible to heat than the old culture. These findings suggest that the resistance to heat of the infecting bacilli in the lungs of the chronically infected mice is a phenomenon indicative of their physiologic state and is not merely a consequence of the presence of lung tissue. They are consistent with the view that the bacilli are in a resting state. The constant level of culturable bacilli obtained in the lungs of chronically infected mice would therefore appear to denote a persistence of nonmultiplying organisms rather than a dynamic equilibrium between multiplication and destruction. This conclusion is in agreement with that of Rees and D'Arcy Hart (7), who compared culturable and total acid-fast counts in the lungs of chronically infected mice, including those used in this work.

This interpretation of the results of the heat-resistance tests in the group of mice with chronic infections receives indirect support from the results obtained with the acutely infected animals. The difference in heat susceptibility of the infecting bacilli in the two groups was small but real, and consistent with the view that the bacilli in chronic infections are in a less active state than those in acute infections.

The failure of the bacilli in the lungs of the "acute" mice to resemble young cultures in susceptibility to heat is not altogether unexpected for two reasons. First, the doubling time of bacilli in the lungs of the "acute" mice is about twenty-seven hours between the tenth and fifteenth days after infection; this is about twice as long as the doubling time of bacilli in 7H-10 medium during the logarithmic phase of growth. Such a difference in speed of division might be accompanied by an appreciable difference in the susceptibility of the bacilli to heat. Second, it is possible that the bacilli in some parts of the lung might have ceased growing, and that the homogenized lung suspensions contained a mixture of multiplying and nonmultiplying organisms. Heating a mixed population might well yield the type of killing curve

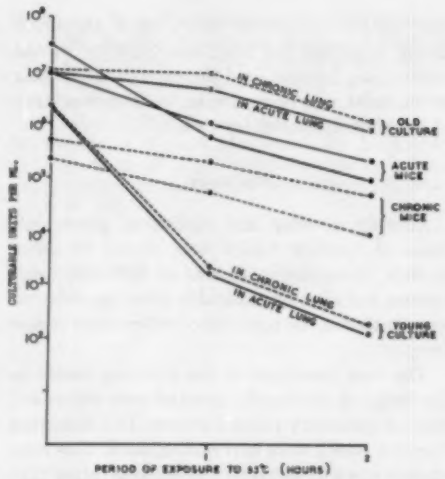

Fig. 4. The heat resistance of the infecting bacilli in the lungs of mice with acute and chronic tuberculosis, and the heat resistance of young and old cultures of H37RvSR when suspended in tuberculous mouse lung.

TABLE 1

Heat Resistance of Infecting Bacilli in Acute and Chronic Tuberculous Infection in the Mouse

Infection in Mice	Initial Culturable Count (Culturable Units per Ml.)	Decrease in Culturable Count during Heating at 53°C. (Log$_{10}$ Culturable Units per Ml.)	
		0–1 Hours	1–2 Hours
Chronic	1.2×10^7	0.42	0.63
	1.6×10^6	0.42	0.79
	8.8×10^5	0.52	0.68
Average	$2.6 \times 10^{6*}$	0.45	0.70
Acute	8.3×10^6	0.98	0.56
	1.3×10^8	1.70	0.73
	1.8×10^7	0.98	0.99
	5.7×10^7	1.80	0.75
	1.8×10^7	1.08	0.31
	9.1×10^6	1.06	0.78
Average	$2.4 \times 10^{7*}$	1.27	0.69

* Geometric mean

that was found with the "acute" mice, the initial killing reflecting the heat susceptibility of the multiplying fraction, and the subsequent slowing of the death rate being due to the heat resistance of the nonmultiplying fraction.

Summary

Actively growing and stationary phase cultures of tubercle bacilli were found to differ in their susceptibility to heat at 53°C. This difference was also demonstrable when the cultures were heated in the presence of tuberculous mouse lung.

The heat resistance of the infecting bacilli in the lungs of chronically infected mice resembled that of stationary phase cultures. This indicated that the bacilli were in a resting state. This conclusion was supported by the demonstration that the bacilli in the lungs of acutely infected mice were more susceptible to heat, although the difference was small.

The constant level of culturable bacilli in the lungs in chronic tuberculous infection in the mouse, therefore, is thought to be due to the persistence of nonmultiplying bacilli rather than to a dynamic equilibrium between multiplication and destruction.

Sumario

La Resistencia Térmica de los Bacilos Tuberculosos en los Pulmones de los Ratones Infectados

Se observó que los cultivos de bacilos tuberculosos en desarrollo activo y en la fase estacionaria discrepaban en su susceptibilidad al calor de 53°C. También era observable esta diferencia cuando se calentaban los cultivos en presencia de pulmón murino tuberculoso.

La termorresistencia de los bacilos infectantes en los pulmones de los ratones infectados crónicamente se parecía a la de los cultivos en la fase estacionaria, lo cual indicaba que los bacilos se hallaban en un estado de reposo. Esta conclusión encontró apoyo en la demostración de que los bacilos de los pulmones de los ratones infectados agudamente eran más susceptibles al calor, aunque era poca la diferencia.

Por lo tanto, el constante nivel de los bacilos viables de los pulmones en la tuberculosis murina crónica parece deberse a la persistencia de bacilos que no se multiplican más bien que a un equilibrio dinámico entre al multiplicación y la destrucción.

Resume

La résistance à la chaleur des bacilles tuberculeux dans les poumons de souris infectées

On trouva que des cultures de bacilles tuberculeux, poussant activement et à la phase de repos, diffèrent dans leur sensibilité à la chaleur à 53°C. Cette différence put aussi être démontrée lorsqu'on chauffa les cultures en présence de poumon de souris tuberculeuses.

La résistance à la chaleur des bacilles infectant, dans les poumons de souris infectées d'une façon chronique, ressemble à celle des cultures à la phase de repos. Cette conclusion fut appuyée par la démonstration que les bacilles dans les poumons de souris infectées d'une façon aiguë furent plus susceptibles à la chaleur, bien que la différence fut minime.

Le niveau constant de bacilles viables dans les poumons de souris tuberculeuses chroniques est, pense-t-on, par conséquent, dû à la persistance de bacilles ne se multipliant pas, plutôt qu'à un équilibre dynamique entre la multiplication et la destruction.

Acknowledgments

The writer is greatly indebted to Dr. D. A. Mitchison for his continued advice and encouragement, and for reading the manuscript; to Dr. R. J. W. Rees and Dr. P. D'Arcy Hart for providing the infected mice and facilities for working in their laboratory; to Miss Janet Lloyd for preparing media; and to Mr. W. Clifford for preparing the figures.

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INCREASE IN CIRCULATING LYSOZYME-LIKE ENZYME FOLLOWING SENSITIZATION OF GUINEA PIGS WITH MYCOBACTERIUM TUBERCULOSIS[1, 2]

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INTRODUCTION

A marked increase in the concentration of a lysozyme-like enzyme in rabbit serum following immunization with heat-killed tubercle bacilli has been reported by Myrvik and Weiser (1). In addition, Kerby and Chaudhuri (2) found that plasma concentrations of a similar substance were significantly higher in tuberculous patients than in nontuberculous subjects. The present writers have found that a similar situation prevails when guinea pigs are sensitized with heat-killed tubercle bacilli. During an earlier study (3), in which the release of a lysozyme-like enzyme from leukocytes of tuberculin-hypersensitive guinea pigs was investigated, it was noted that concentrations of the enzyme in plasma were usually higher in sensitized guinea pigs than in control animals. The purpose of the present report is to further describe the increase in the lysozyme-like enzyme circulating in tuberculin-sensitive guinea pigs and to suggest possible mechanisms which may influence the concentration of enzyme.

MATERIALS AND METHODS

Sensitization and skin testing: Normal, male, albino guinea pigs, weighing approximately 350 gm., were rendered hypersensitive to tuberculin by the intramuscular injection of 1 ml. of sterile paraffin oil containing 5 mg. (dry weight) of heat-killed human tubercle bacilli (H37Rv).[4] The bacilli were harvested from autoclaved six-week-old cultures on modified Proskauer and Beck liquid medium (4). The bacillary mass was filtered, washed with distilled water, and dried at 110°C. for one hour. For animal injection, the bacilli were suspended in paraffin oil and sterilized in the autoclave. Tuberculin hypersensitivity was determined by intradermal injection of 5γ of PPD.[5]

Enzyme assay: Whole blood was obtained by cardiac puncture from animals that were fasted overnight. Serum or plasma was collected and stored without preservatives at −20°C. for subsequent study. Assays of the lysozyme-like enzyme were performed in duplicate with 0.1-ml. aliquots of either serum or plasma. To each sample were added 0.9 ml. of M/15 phosphate buffer (pH 7.0) and 1.5 ml. of a 200-mg. per 100 ml. suspension of Bacto-Lysozyme substrate[6] in the phosphate buffer. The optical density of the suspensions was read immediately with an electrophotometer[7] at 525 mμ, and again after twenty minutes' incubation at 37°C. The instrument was standardized with a tube containing 2.5 ml. of buffer. The per cent lysis of the substrate was calculated for each sample as the difference between the initial and final optical densities divided by the initial optical density. The per cent lysis of the substrate was converted to lysozyme equivalents by reference to a standard curve which had been prepared previously using purified egg-white lysozyme.[8]

Cytolysis test: Tuberculin leukocytolysis was determined by a method based on the release of a leukocytic, lysozyme-like enzyme. The technique was found previously (3) to be an acceptable substitute for microscopic leukocyte counts. Four siliconized serology tubes, each containing 0.8 ml. of heparinized whole blood, were prepared for each blood sample. Two-tenths of a milliliter of pyrogen-free saline[9] was added to each of two tubes; each of the two remaining tubes received 0.2 ml. of saline which contained 10γ of PPD. The tubes were sealed with silicone stoppers, rotated on a roller drum at 37°C. for two hours, and centrifuged. Enzyme assays were performed on 0.1-ml. aliquots of the cell-free supernatant fluids, as described above. Tuberculin cytolysis was recorded as the lysozyme Δ, which represents the difference between the per cent lysis of the substrate obtained with the tubes containing PPD and those to which only saline was added.

Statistical analysis: The methods outlined by Croxton (5) were used for analysis of the sig-

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[2] Presented in part before the Medical Session, as part of *Section 3B*, at the annual meeting of the American Trudeau (now Thoracic) Society, Chicago, Illinois, May 26, 1959.

[3] Part of this material was contained in a thesis submitted to The George Washington University, Washington, D. C., in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[4] Culture originally obtained from Dr. William Steenken, Jr., Saranac Lake, New York.

[5] Merck, Sharp, and Dohme.

[6] Difco Laboratories, Detroit, Michigan.

[7] Fisher Scientific Co., Washington, D.C.

[8] Baxter Laboratories, Morton Grove, Illinois.

nificance of differences between means and for analysis of linear correlation.

Results

In a preliminary experiment, the concentration of lysozyme-like enzyme in the serum of 12 normal guinea pigs was compared with the values in 12 guinea pigs that had been injected four months previously with heat-killed tubercle bacilli in paraffin oil. The results are shown in table 1. The concentration of enzyme was significantly ($P < 0.01$) elevated in the serum of the sensitized animals.

Subsequent experiments were designed to determine at what time following injection of tubercle bacilli the increase in enzyme first becomes demonstrable.

In the first experiment, 36 normal guinea pigs were injected with heat-killed tubercle bacilli suspended in paraffin oil. Four animals were bled for serum immediately prior to injection, and the remaining animals were bled for serum in groups of 4 at various intervals after injection. A second experiment was performed in the same manner except that plasma was collected instead of serum.

The results are summarized in table 2, in which each value represents the mean of samples from 4 animals. The results of these experiments indicate that a highly significant increase ($P < 0.01$) in serum and plasma concentration of lysozyme-like enzyme occurs soon after injection of guinea pigs with heat-killed tubercle bacilli. The increase in enzyme occurred between seven and ten days in the first experiment, and between eleven and fourteen days in the second experiment. Animals injected with paraffin oil alone showed no significant alteration in the concentration of enzyme.

TABLE 1

Concentration of Lysozyme-like Enzyme in Serum of Normal and Tuberculin-Sensitive Guinea Pigs

Lysozyme Equivalents	
Normal	Sensitive
γ per ml.	
3.6	7.7
3.8	7.9
3.8	7.5
6.2	6.6
3.8	7.0
5.4	7.0
4.8	7.6
3.5	10.1
4.9	7.2
5.2	10.0
6.1	8.0
5.2	9.1
Mean 4.7	8.0

TABLE 2

Serum and Plasma Concentrations of Lysozyme-like Enzyme following Injection of Guinea Pigs with Heat-killed Tubercle Bacilli

Days after Injection	Mean Lysozyme Equivalents	
	Experiment 1 (Serum)	Experiment 2 (Plasma)
	γ per ml.	
0	5.0	4.2
3	5.7	—
4	—	4.4
5	6.2	—
7	5.8	4.9
9	—	5.4
10	7.9	—
11	—	5.7
12	7.4	—
14	7.7	7.7
17	7.7	—
18	—	8.1
21	7.2	—
25	—	7.9
32	—	10.5

— = Not done

The increase in enzyme seemed to occur at approximately the same time that skin sensitivity to tuberculin (6) and leukocytic susceptibility to lysis *in vitro* by PPD (3) developed. An experiment was performed to examine this possibility.

Twenty-four normal guinea pigs were injected with heat-killed tubercle bacilli and bled in groups of 4 at various intervals after injection. Whole blood was collected for the leukocytolysis tests and a plasma sample was separated and retained for enzyme assay. The animals were tested intradermally with PPD immediately after bleeding.

The results of the cytolysis tests, PPD skin tests, and assays for lysozyme-like enzyme are presented in table 3. Each value represents the mean of 4 animals. The experimental values were compared with values obtained from 30 normal guinea pigs which showed means of +0.7 for the PPD lysozyme Δ, and 3.9γ per ml. for plasma concentrations of lysozyme-like enzyme. On this

TABLE 3

DEVELOPMENT OF PPD LEUKOCYTOLYSIS AND PPD SKIN SENSITIVITY IN COMPARISON WITH THE INCREASE OF LYSOZYME-LIKE ENZYME IN THE PLASMA FOLLOWING INJECTION OF GUINEA PIGS WITH HEAT-KILLED TUBERCLE BACILLI

Days after Injection	Mean PPD Lysozyme Δ	Mean PPD Skin Reaction*	Mean Lysozyme Equivalents
			γ per ml.
5	−0.1	1.0	5.2
8	+2.3	4.1	5.1
12	+1.5	4.7	7.2
15	+1.5	7.1	6.1
19	+2.9	7.2	7.6
26	+4.8	11.2	8.6

* Millimeters of induration at 48 hours

basis, PPD cytolysis was significant ($P < 0.01$) at nineteen and twenty-six days, and plasma concentrations of enzyme were significantly elevated ($P < 0.01$) at twelve, fifteen, nineteen, and twenty-six days. Significant skin sensitivity to tuberculin appeared between twelve and fifteen days, and persisted. Although skin hypersensitivity to tuberculin, leukocytic susceptibility to lysis by PPD, and plasma concentrations of lysozyme-like enzyme did not reach statistical significance at the same time interval, these parameters exhibited a similar increasing trend during the first month of sensitization.

The next experiments were designed to determine whether, in tuberculin-hypersensitive guinea pigs, there is any correlation between the amount of plasma enzyme, skin reactivity to tuberculin, and *in vitro* tuberculin leukocytolysis. A group of 23 guinea pigs which had been injected four months previously with heat-killed tubercle bacilli were bled for plasma and skin tested with PPD immediately after bleeding. The tuberculin reactions and plasma enzyme concentrations are shown in table 4. Analysis of these results showed no linear correlation ($r = -0.05$). In a similar experiment, the values for lysozyme-like enzyme in the plasma were compared with the degree of *in vitro* PPD leukocytolysis in another group of 23 guinea pigs that had been sensitized approximately four months previously. The results are presented in table 5. In this case, as with the PPD skin reactions, the correlation coefficient (0.16) was not significant.

The effect of a tuberculin skin test on serum and plasma concentrations of lysozyme-like enzyme also was investigated.

In the first experiment, 16 guinea pigs which had been sensitized with heat-killed tubercle bacilli four months previously were divided into four groups and, immediately before skin testing with PPD, one group was bled for serum. Subsequently, a group was bled for serum at one, two, and three days after skin testing. The second experiment also was performed with 16 guinea pigs that had been injected four months previously with heat-killed tubercle bacilli. However, plasma, instead of serum, was collected at zero, two, four, and eight days following the skin test.

The results are summarized in table 6, in which each value is the mean of 4 animals. No significant alteration in enzyme concentration was observed in either experiment. Indurated skin reactions with average diameters of 13.7 mm. in the first experiment and 10.2 mm. in the second experiment were observed at forty-eight hours after skin testing. Approximately one-half of the animals in each experiment exhibited central necrotic areas at forty-eight hours. Nor-

TABLE 4

COMPARISON OF PLASMA CONCENTRATIONS OF LYSOZYME-LIKE ENZYME AND PPD SKIN SENSITIVITY IN TUBERCULIN-SENSITIVE GUINEA PIGS APPROXIMATELY FOUR MONTHS AFTER INJECTION OF HEAT-KILLED TUBERCLE BACILLI

Animal Number	PPD Skin Reaction*	Lysozyme Equivalents
		γ per ml.
826	7.0	6.2
831	8.5	10.1
832	8.5	8.9
827	9.5	9.2
829	9.5	7.1
837	10.0	9.4
830	11.0	8.2
834	11.0	10.5
836	11.0	7.0
833	11.5	6.4
835	12.0	7.5
186	12.0	7.5
188	12.0	7.0
189	12.0	7.0
828	13.0	7.7
184	13.0	7.7
42	13.0	9.1
185	14.0	7.9
187	14.0	6.6
190	14.0	7.6
193	14.0	10.0
191	15.0	10.1
192	18.0	7.2

* Millimeters of induration at 48 hours

TABLE 5

COMPARISON OF PLASMA CONCENTRATIONS OF LYSOZYME-LIKE ENZYME AND IN VITRO PPD LEUKOCYTOLYSIS IN TUBERCULIN-SENSITIVE GUINEA PIGS APPROXIMATELY FOUR MONTHS AFTER INJECTION OF HEAT-KILLED TUBERCLE BACILLI

Animal Number	PPD Lysozyme Δ	Lysozyme Equivalents
		γ per ml.
507	+0.4	6.5
522	+3.8	6.6
405	+3.8	5.6
83	+3.9	6.5
48	+4.3	7.8
520	+5.5	7.5
94	+5.6	7.5
41	+6.4	5.4
428	+6.8	6.7
504	+6.8	6.3
508	+6.8	6.4
524	+7.2	5.1
76	+7.2	7.4
430	+8.4	9.9
98	+8.6	6.3
86	+8.7	5.6
511	+9.9	6.0
412	+10.9	7.2
38	+11.3	5.7
84	+12.7	6.4
96	+13.0	9.0
406	+13.2	6.3
600	+16.2	7.5

mal animals that were skin tested with PPD also showed no alteration in the amount of enzyme.

DISCUSSION

To the writers' knowledge, the elevation of lysozyme-like enzyme in the serum and plasma of guinea pigs injected with heat-killed tubercle bacilli in paraffin oil has not been reported previously. However, as mentioned earlier, a similar enzyme alteration has been observed in rabbits sensitized with heat-killed tubercle bacilli (1) and in tuberculous humans (2). The elevation of enzyme in sensitized guinea pigs occurred within one to two weeks after injection of tubercle bacilli (*see* tables 2 and 3) and seemed to persist for some time, even as long as four months, after sensitization.

The actual mechanism responsible for the increase in the enzyme following sensitization is not known. However, since the leukocyte is considered to be a primary source of the enzyme (7), one might suspect that some form of leukocyte damage or lysis might be responsible for the increase. The possible role of leukocyte trauma which may occur *in vitro* during collection and centrifugation of blood cannot be ignored in this respect. Flanagan and Lionetti (8) demonstrated that variation of blood anticoagulation techniques may affect the amounts of lysozyme present in plasma. In the present study, however, the clotting process did not appear to play a role in the increase in enzyme following sensitization since similar enzyme elevations were observed in both plasma and serum (*see* table 2). To attribute the increase in enzyme to an artifact arising from leukocyte damage *in vitro* it would necessarily follow that, during sensitization, leukocytes of sensitized guinea pigs become more susceptible to damage during blood collection and centrifugation. Although this possibility was not explored in the present study, it is of interest to mention Patnode and Hudgins' finding (9) that monocytes from guinea pigs injected with heat-killed H37Rv organisms are more resistant to disruption by the mechanical trauma of sonic vibration than are similar cells of normal animals. However, one cannot necessarily conclude that leukocytes which are resistant to sonic vibration are also resistant to other forms of trauma.

The possibility exists that damage or lysis of leukocytes *in vivo* may cause an increased concentration of lysozyme-like enzyme following sensitization. The cytotoxic and cytolytic effects of tuberculin on leukocytes of sensitive animals might be considered in this respect. However, the experimental results do not support this hypothesis. Although an increase in en-

TABLE 6

EFFECT OF TUBERCULIN SKIN TEST ON CONCENTRATION OF LYSOZYME-LIKE ENZYME IN TUBERCULIN-SENSITIVE GUINEA PIGS

Days after Skin Test	Mean Lysozyme Equivalents	
	Experiment 1	Experiment 2
	γ per ml.	
0	8.0	8.6
1	7.6	—
2	8.6	7.6
3	6.4	—
4	—	8.4
8	—	8.6

— = Not done

zyme was found during the development of skin hypersensitivity to tuberculin and leukocytic susceptibility to lysis by PPD soon after sensitization (*see* table 3), a cause-and-effect relationship could not definitely be established. Approximately four months after sensitization no correlation was found between the amount of enzyme in the plasma and either skin reactivity to PPD or PPD leukocytolysis (*see* tables 4 and 5).

The results of experiments concerned with the effect of tuberculin skin testing on the concentration of lysozyme-like enzyme are of interest. It has been suggested (10, 11) that some form of *in vivo* leukocyte lysis by tuberculin may occur during the development of a skin reaction to tuberculin. The results of the present study (*see* table 6) showed that no alteration in the amount of enzyme occurred within eight days after tuberculin skin testing, suggesting that *in vivo* leukocyte damage by tuberculin does not occur. The possibility exists, however, that some leukocyte injury actually may have taken place, but it was of such a low degree that it was not detectable by the assay method used.

In addition to the possible effects of tuberculin on leukocytes *in vivo*, it seems reasonable to believe that increased amounts of enzyme following sensitization may be a reflection of nonspecific leukocyte trauma *in vivo*, which may not be related to tuberculosis or to the leukocytic reactions characteristic of tuberculin hypersensitivity. A rise in serum enzyme has been observed (1) in rabbits injected with heat-killed *Escherichia coli, Saccharomyces cerevisiae*, or *Mycobacterium smegmatis* suspended in paraffin oil. Increased enzyme values also have been found in cancer patients (12) and in mice that have been injected with zymosan (13). Recently, Myrvik (14) suggested that elevated serum lysozyme may be associated with granulomatous inflammation. The possible role of leukocytotoxic substances also should be considered. Using the release of lysozyme-like enzyme as an index of cellular damage, Kerby (15) demonstrated that derivatives of various bacteria were toxic for human leukocytes. The present writers have found (16) that certain lipid constituents of virulent tubercle bacilli (H37Rv) cause the release of enzyme from normal guinea pig leukocytes.

Although the actual mechanism responsible for elevation of the lysozyme-like enzyme in guinea pigs injected with heat-killed tubercle bacilli is not known, the very fact that the enzyme is elevated is of interest. Melsom and Weiser (17) increased the resistance of mice to pneumococcal infection by treatment with lysozyme. Similar attempts by Corper (18) and Weiser and his associates (19) to increase resistance to tuberculosis or to treat the disease with lysozyme have not been successful even though the enzyme has been shown to possess bacteriostatic (20) and bactericidal (21) activity against tubercle bacilli. Despite its failure to date in therapeutic trials, Weiser (22), Seibert (23), and Myrvik (14) have suggested that the enzyme may have an important role in the immune response in tuberculosis.

Summary

The circulating concentrations of a lysozyme-like enzyme in guinea pigs injected with heat-killed tubercle bacilli (H37Rv) suspended in paraffin oil were significantly higher than those in normal animals. The increase in enzyme was found to occur early in the course of sensitization and to persist as long as four months, at which time there appeared to be no correlation between the amount of enzyme in the plasma and either skin hypersensitivity to tuberculin or susceptibility of leukocytes to lysis by PPD *in vitro*. Tuberculin skin testing of sensitized animals produced no significant alterations in the concentration of enzyme. Various mechanisms which might be responsible for elevated enzyme values following sensitization are discussed.

Sumario

Elevación de las Concentraciones Circulantes de un Enzima Lisozimoideo a Continuación de la Sensibilización de los Cobayos con Mycobacterium Tuberculosis

Las concentraciones de un enzima lisozimoideo en la circulación de los cobayos a los que se inyectaron bacilos tuberculosos (H37Rv) matados al calor y suspendidos en aceite de parafina resultaron ser significativamente más altas que las de animales normales. Se observó que el aumento del enzima tenía lugar tempranamente en el transcurso de la sensibilización y que persistía aun a los cuatro meses, para cuya fecha no parecía que hubiese correlación entre las concentraciones del enzima en el plasma y ya la cutihipersensibilidad a la tuberculina o la susceptibilidad de los leucocitos a la lisis por PPD (DPP) *in vitro*. La comprobación cutánea con tuberculina en los animales

sensibilizados no produjo alteraciones de importancia en las concentraciones de enzima. Se discuten varios mecanismos que podrían ser la causa de la elevación de las concentraciones de enzima consecutivamente a la sensibilización.

Resume

Elévation des niveaux d'un enzyme semblable àu lysozyme en circulation après sensibilisation de cobayes à Mycobacterium tuberculosis

Les niveaux d'un enzyme en circulation, semblable au lysozyme, chez des cobayes à qui on a injecté des bacilles tuberculeux tués par la chaleur (H37Rv), en suspension dans de l'huile de paraffine, furent nettement plus élevés que ceux d'animaux normaux. On trouva que l'augmentation en enzyme se produit tôt au cours de la sensibilisation et persiste aussi longtemps que quatre mois, moment où il semble qu'il n'y a pas de corrélation entre les niveaux d'enzyme dans le plasma et l'hypersensibilité cutanée à la tuberculine ou la sensibilité des leucocytes à la lyse par PPD *in vitro*. La réaction cutanée à la tuberculine des animaux sensibilisés ne produisit pas de changement important dans les niveaux d'enzyme. Les auteurs discutent des méchanismes variés qui pourraient être responsables des niveaux élevés d'enzyme après sensibilisation.

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Case Reports

PULMONARY ALVEOLAR PROTEINOSIS[1]

Report of a Case in Japan

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INTRODUCTION

In 1958, Rosen, Castleman, and Liebow (1) described a new clinicopathologic entity called "pulmonary alveolar proteinosis." They collected 27 studies of this disease. Since then three cases have been reported by Sieracki, Horn, and Kay (2), one case by Moertel, Woolner, and Bernatz (3); one by Landis, Rose, and Sternlie (4); one by Payseur, Konwaler, and Hyde (5); one by McDowell, Williams, and Hinds (6); one by Carabasi and Barta (7); one by Pitt (8); one case by Carlson and Mason (10); and four cases by Plank, Swift, Chambers, and Peltzer (11).

Although the histologic features were characteristic and similar in these reports, the clinical patterns varied somewhat in each case. The present case, probably the first detected in Japan, is reported in detail, because the patient apparently had no complaints and had detailed cardiopulmonary function studies, which may contribute to the clinical picture of the disease.

CASE REPORT

A 19-year-old female entered the Second Internal Clinic of Iwate Medical College on November 11, 1959. In the summer of 1958, her disease was diagnosed as pulmonary tuberculosis in a roentgenographic survey (figure 1). At that time, she had no symptoms except slight fatigue. Before admission, the patient had been treated with streptomycin, PAS, and isoniazid for two months and then with PAS and isoniazid for twelve months in a local hospital in Iwate Prefecture, but there was no apparent response to these drugs. The roentgenogram obtained in November, 1959, at this Clinic was considered to be consistent with sarcoidosis (figure 2).

Past history: The tuberculin reaction was known to have become positive when the patient was eleven years old. She had sometimes had a feeling of catching a cold, with slight dry cough in the winter season; but chest films obtained in routine mass school surveys once a year from the ages of nine to fifteen years were considered normal. In March, 1957, the patient went to work in Yokohama as a worker in a transistor manufacturing plant where, for one year, she handled fine strings as well as an adhesive "Semedain" containing amyl nitrite, ethyl amyl, and phenol formaldehyde.

Physical examination: The patient was well developed and fairly well nourished. Her height was 159.5 cm.; her weight, 53 kg. Her pulse was 72 per minute and the temperature, 36.5°C. Sclerae and conjunctivae were normal, with no jaundice or pallor. The patient showed no evidence of cyanosis or clubbing of the fingers. The skin was warm, and there was no evidence of jaundice, ecchymosis, eruption, or pigmentation. No palpable lymphadenopathy was noted. Her blood pressure was 108 systolic, 80 diastolic in mm. of mercury. The electrocardiogram was normal. The tuberculin reaction was positive. Sputum was negative for acid-fast bacilli and fungi by culture as well as by microscopy.

A roentgenogram of the chest showed diffuse nodular densities with prominence of both hilar areas and of both basal lung fields (figures 1, 2, 3). Planigrams revealed no cavity. Calcified foci or enlargements of hilar nodes were not observed (figure 4). Bronchograms showed no pathologic abnormalities (figure 5).

Laboratory data: The erythrocyte count was 5,720,000 per cu. mm.; the hematocrit, 49.9 per cent. The hemoglobin was 19 gm. per 100 ml. The leukocyte count was 6,000 per cu. mm. The differential leukocyte count in per cent was: neutrophils, 57; lymphocytes, 33; eosinophils, 3, monocytes, 7. On November 14, 1959, the erythrocyte count was 4,640,000 per cu. mm. Two months later (January 16, 1960) the hemoglobin was 14.39 mg. per 100 ml.; the leukocyte count, 6,600 per cu. mm. with 71 neutrophils, 2 eosinophils, 25 lymphocytes, and 2 monocytes. The erythrocyte sedimentation rate was 5 mm. in one hour on November 13, 1959, and 5 mm. on January 12, 1960.

Liver function studies: The icterus-index, Takada's reaction, or cerebrospinal fluid, were within normal limits (November 16, 1959). The total

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FIG. 1 (Upper). Roentgenogram of September 12, 1958, taken soon after the film obtained during the mass roentgenographic survey. Diffuse nodular infiltration with prominence of both hilar areas and both basal lung fields are seen. A few nodular densities are in the left lower fields and also in the first and fourth right intercostal spaces. Cavity, hilar lymph node enlargement, and calcified foci are not found.

FIG. 2 (Center). Roentgenogram of November 10, 1959, at the time of the patient's visit to the Clinic. Before then she had been treated with streptomycin, PAS, and isoniazid for two months; then with PAS and isoniazid for one year. The roentgenographic finding is nearly the same as in figure 1.

FIG. 3 (Lower). Roentgenogram of January, 1960, taken before biopsy was performed.

serum protein was 8.3 gm.; albumin, 50.9 per cent; α-globulin, 16.5 per cent; β-globulin, 9.3 per cent; γ-globulin, 22.8 per cent; serum sodium, 144.0 mEq/L.; serum potassium, 4.7 mEq/L.; 17 ketosteroids in urine were 3.2 mg. per day, and 17 ketosteroid hydroxy corticosteroids (OHCS) in urine 3.9 mg. per day. Serum cholesterol was 184 mg. per 100 ml.

As shown in table 1, the ventilatory function and intrapulmonary gas distribution were absolutely normal, except for a slight increase of residual volume. This increase of residual volume may be considered as a compensatory overdistention of the remaining normal lung because the patient had no sign of obstruction. The most characteristic finding was a decrease of carbon monoxide pulmonary diffusing capacity determined by a single-breath method; this was interpreted as a slight evidence of alveolar-capillary block in this patient. However, the increase of diffusing capacity for carbon monoxide (D_{CO}) during exercise was quite remarkable, showing that the expansibility of the pulmonary capillary bed in this patient was well reserved. As the walking index was normal and the patient had no dyspnea during exercise, the change of alveolar capillary block was thought to be of slight degree.

The results of right heart catheterization are shown in table 2. Arterial oxygen saturation was 91.6 per cent and oxygen pressure (P_{O_2}) was 63 mm. of mercury showing a slight decrease, but other data were within normal limits.

Roentgenographic examination of the family: The patient's parents, one brother, and one sister were examined roentgenographically, but they showed no abnormalities in their chest films.

After admission the patient had no complaints. Her temperature, pulse, and respiration were normal. She was treated with a triple-drug regimen: 1.0 gm. of streptomycin, intramuscularly, twice a week; 150 mg. of isoniazid twice a week; and 10 gm. of PAS daily: but there was no roentgenographic evidence of response to these drugs.

On February 2, 1960, a biopsy of the right middle lobe was performed.

Macroscopic findings: On the cut surface of the resected specimen of the right middle lobe were discrete, sharply defined lesions, in which multiple small nodular, yellow-gray, pasty-film

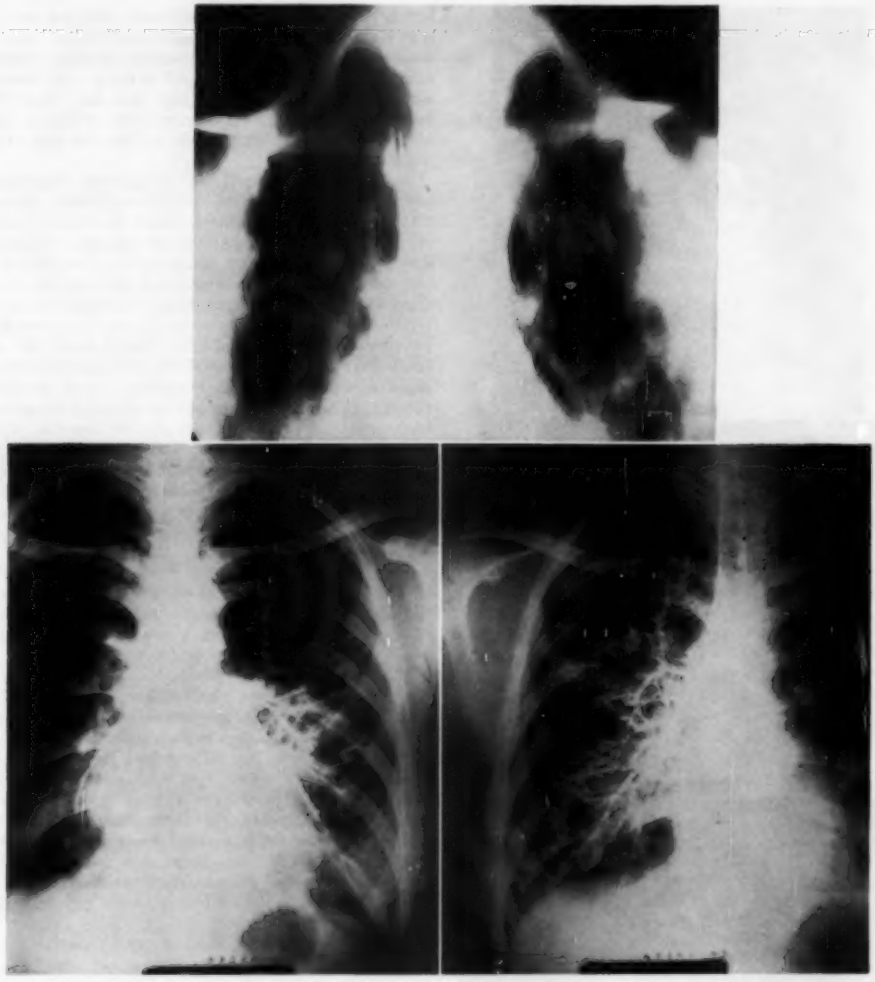

FIG. 4 (Upper). Planigram of November 19, 1959. There are a few nodular densities in the left middle and lower lung fields and also in the right upper and right middle fields. No cavity is present.

FIG. 5*A* (Lower left). Bronchogram of right side of chest, obtained on November 26, 1959, revealed no pathologic changes.

FIG. 5*B* (Lower right). Bronchogram of left side of chest, obtained on December 1, 1959, nearly normal.

substances were noticeable (S_4 and S_5). There were no signs of pleuritis.

Histologic findings: Paraffin sections of the tissues obtained from several parts of the involved lung, fixed with either formalin or absolute alcohol, were stained with hematoxylin and eosin. The amorphous eosinophilic substance, the so-called "proteinaceous" material, filled most of the alveoli. The material in the alveoli caused marked thinning or even ruptures of the septa in some places, but there were no inflammatory changes. On the other hand, it was noted that the cells lining alveolar septa in some parts were multilayered, apparently as a

TABLE 1

PULMONARY FUNCTION TESTS

Sex: F Age: 18 Date: January 29, 1960
Weight: 53 kg. Height: 159.5 cm.
Body Surface Area: 1.54 M^2

	Observed	Normal	Per Cent Normal
Maximal breathing capacity (L./min.)	106.6	97	110
Walking ventilation (L./M^2 min.)	12	8–11	
Walking index	17%	25%	
Dyspnea	(–)		
Vital capacity	3,412	3,200	114
Timed vital capacity: 1 second	93	75%	
2 seconds	98	85%	
3 seconds	100	95%	
Air velocity index	0.96	1.00 ± 0.2	
Spirometry and Lung Volume			
	Observed	**Normal**	**Per Cent Normal**
Respiratory rate/minute	9		
Tidal volume, cc.	592		
Inspiratory capacity, cc.	2,619		
Expiratory reserve, cc.	793		
Vital capacity, cc.	3,412	3,200	114
Minute ventilation, liters	5.3		
Minute oxygen consumption, cc.	226		
Ventilatory equivalent	2.36		
Residual volume	1,179	800	147
Total lung capacity	4,591	4,000	115
RV/TLC ratio	25.7	20.0	

D_{CO} at rest 21.91 cc./min./mm. Hg. (71% of predicted).
D_{CO} during exercise 27.30 cc./min./mm. Hg.

result of proliferation (figure 6). Arginophil fibers were increasing in the thickening wall of septa. There was no inflammatory reaction between the normal and the involved alveoli. The epithelia of small bronchioles were not affected when the proteinaceous substance appeared. The cells lining alveolar septa, "septal cells," in some parts were in process of desquamation in alveoli (figure 7). These cells were found to be foamy, degenerated, and sloughed in the lumen, showing signs of karyorrhexis and necrosis of nuclei (figure 8).

The "laminated" bodies, described by Rosen and others (1), however, could not be found in this specimen. The amorphous substance was strongly positive with periodic acid-Schiff stains as well as with the Best-carmine reagents, even after a salivary digestion. This intra-alveolar substance was metachromatic and was also positive with Feulgen reaction. No fluorescence was observed in the proteinaceous substance with auramine staining. The substance appeared light green with Masson-Goldner's modification of trichrome, and generally gave a blue tint with Azan-Mallory or Weigert's fibrin stains. The muci-carmine reaction was generally negative, although both the epithelium of the bronchioles and a point of material in the lumen gave slightly positive results. Crystal-violet and Congo-red reactions for amyloid were negative.

Sudan III, Sudan black B, and Oil red-O staining for this substance showed only a small collection of lipid in the degenerated large mononuclear cells. Some of the lumina showed cholesterin crystal refracted to the polarized light. The proteinaceous substance was not found positive for cholesterin with both the Schultz and Smith-Dietrich reactions.

Findings of microorganisms with various stainings, such as the Gram, the MacCallum-Goodpasture, the Ziehl-Neelsen, and the Giemsa stains, gave negative results.

TABLE 2

DATA ON RIGHT CARDIAC CATHETERIZATION

		Observed Value	Normal Value
Hematocrit		49.0	(39–52)
Right atrial pressure		2.2 mm.Hg	(−2–2)
Right ventricular pressure	Systolic	27.5 mm.Hg	(27.25) (22.25)
	Diastolic	−1 mm.Hg	(5.75) (0.5)
	Mean	10.0 mm.Hg	(11.0)
Pulmonary arterial pressure	Systolic	26.0 mm.Hg	(25.0)
	Diastolic	6 mm.Hg	(8.0)
	Mean	15.1 mm.Hg	(15.0)
Pulmonary capillary pressure (mm.Hg.)		8.0	(6.0)
Difference between pulmonary and capillary pressure (mm.Hg.)		17.1	(9.0)
Pulmonary arterial resistance (dyne/sec./cm.$^{-5}$)		103.4	(70.0)
Total pulmonary vascular resistance (dyne/sec./cm.$^{-5}$)		219.8	
Bronchial arterial pressure	Systolic	112	(120.0)
	Diastolic	76	(80)
	Mean	88	(93)
Systolic peripheral resistance (dyne/sec./cm.$^{-5}$)		1,252	
Total peripheral resistance (dyne/sec./cm.$^{-5}$)		1,281	(1,200)
A-M V_{O_2} diff (volume per cent)		3.91	(4)
O_2 consumption (cc./min.)		214.7	(300)
Arterial blood	O_2-volume of arterial blood (volume per cent)	20.48	(20–21)
	O_2 saturation	91.6	(95–97)
	P_{O_2} mm.Hg.	63	(75–100)
	P_{CO_2} mm.Hg.	33	

	Observed Value	Normal Value
O_2 content of mixed venous blood (volume per cent)	14.68	(13–15)
O_2 saturation (per cent)	72.6	
Cardiac output (L./min.)	5.491	(4.81 ± 0.65)
Cardiac index (L./min./m^2)	3.566	(3.21 ± 0.76)
One-stroke volume (cc.)	80.7	(65.5 ± 9.6)
Pulse rate	68/min.	
pH of arterial blood	7.45	(7.38–7.41)
CO_2 (volume per cent)	41.71	(18.73 m mol)
(B_B^+) b mEq/L.	48	(53–45)
pH of mixed venous blood	7.45	
CO_2 (volume per cent)	45.39	(20.38 m mol)
PCO_2 (mm.Hg.)	36.5	
(B_b^+) b mEq/L.	49	

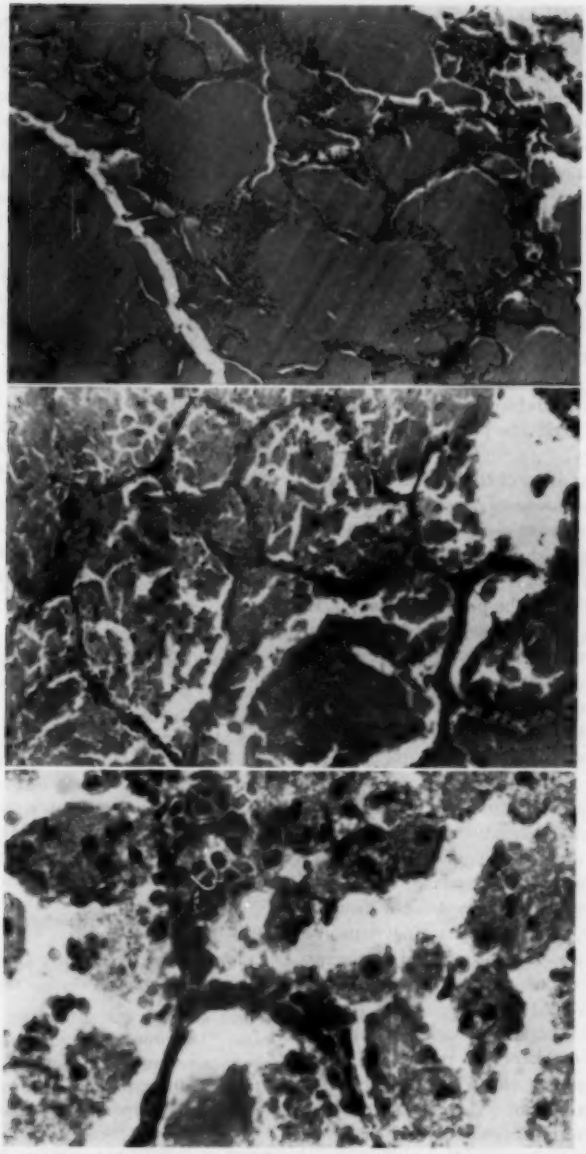

FIG. 6 (Upper). The cells lining alveolar septa are multilayered, apparently as a result of proliferation.

FIG. 7 (Center). "Septal cells" are in process of desquamation and transformation into proteinaceous material.

FIG. 8 (Lower). "Septal cells" are seen in proliferation and desquamation into alveoli. Nuclei are in process of carioclasis, pyknosis, or necrosis.

In the bronchial lymph nodes, hyperplasia of reactive centers was noted, and some of the proliferated endothelial cells were found to be positive with periodic acid-Schiff stains.

Discussion

Pulmonary alveolar proteinosis is a rare disease, and the etiology is still unknown. However, suggestions as to its etiology have been made by Payseur and others (5). They suggest that inhaled pulmonary irritant may cause this condition. The patient was working in a transistor manufacturing plant and was using a chemical material which contained amyl nitrite, ethyl amyl, and phenol formaldehyde. These materials are quite irritant. Therefore, in this case, the inhaled pulmonary irritants may have played a part in the etiology.

Characteristic findings of the clinical picture of this disease are an insidious onset of progressive dyspnea, cough, frequent chest pain, fatigue and, later, loss of weight, clubbing, and cyanosis. In fact, dyspnea was noted in twenty-two of thirty-six cases reported up to the present. However, in the present case, the disease was detected by mass roentogenographic survey, and the patient had none of these complaints or clinical symptoms, not even dyspnea. This patient showed nearly normal pulmonary function except for a slight impairment of pulmonary diffusing capacity.

Concerning pulmonary function studies of this disease, Rosen and associates (1), Sieraki and co-workers (2), and Nagahama (9) also have made reports. Rosen and associates studied 10 cases and reported that usually a slight decrease in vital capacity, maximal breathing capacity, and arterial oxygen saturation, and a moderate increase in alveolar and arterial oxygen difference appeared in two cases, which suggest evidence of alveolar capillary block.

Sieraki and co-workers (2) reported a slight restrictive insufficiency and a slight decrease of arterial oxygen saturation. Nagahama (9) found a slight restrictive insufficiency with a slight decrease of vital capacity, residual volume, and —the most significant finding—a marked decrease of pulmonary diffusing capacity. In the present case, the pulmonary function was nearly normal, except for a slight decrease of pulmonary diffusing capacity, although the patient complained of no dyspnea. It was quite remarkable that ventilatory functions were nearly normal despite extensive pulmonary disease with alveolar filling of proteinaceous material. This may be due to the compensatory distention of the remaining normal lung since the progress of this disease was quite slow in this patient.

According to the extent and stage of the disease the pulmonary function may show various impairments from a slight restrictive insufficiency to a severe diffusion impairment, with alveolar capillary block. Pathologic findings of the resected specimen were essentially the same as in Rosen's original report (1).

Summary

A case of the rare disease, "pulmonary alveolar proteinosis," the first observed in Japan, was studied. The patient, detected by mass roentgenographic survey, showed an extensive pulmonary lesion, but no subjective symptoms at all. Cardiopulmonary function was studied in detail, and a decrease of pulmonary diffusing capacity was the characteristic finding.

Sumario

Proteinosis Alvéolo-Pulmonar. Presentación de Un Caso del Japón

Se estudia un caso de la rara enfermedad, "proteinosis alvéolo-pulmonar," el primero del Japón. El enfermo, descubierto en una encuesta radiográfica colectiva, reveló una extensa lesión pulmonar, pero sin ningún síntoma subjetivo. Se estudió a fondo la función cardiopulmonar y la disminución de la capacidad difusora pulmonar fué el hallazgo típico.

Resume

Protéinose pulmonaire alvéolaire. Rapport d'un cas au Japon

Les auteurs ont étudié un cas de la maladie rare, "La protéinose pulmonaire alvéolaire," le premier cas au Japon. Le malade, dépisté par une étude radiographique en masse, montrait une lésion pulmonaire étendue, mais aucun symptôme.

On étudia en détail la fonction cardiopulmonaire, et la donnée caractéristique fut une diminution de la capacité pulmonaire de diffusion.

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PRIMARY PULMONARY ASPERGILLOSIS[1]

With Invasion of the Mediastinal Contents and Lymph Nodes

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Introduction

Fungi of the genus Aspergillus are widespread and are encountered frequently as secondary invaders or accidental contaminants (1). A diagnosis of primary aspergillosis of the lungs is difficult to document, and only a few cases have been reported. The following case report demonstrates some features of aspergillar infection that are quite unusual; a similar case has not been found in the medical literature.

Case Report

The patient was a 25-year-old carpenter and farmer. In 1947, an area of increased infiltration in the left lung was visualized on a routine survey roentgenogram. Although the patient remained asymptomatic, roentgenograms repeatedly showed progressive infiltration, and by 1952, two exploratory thoracotomies (performed elsewhere) demonstrated a nonresectable lesion involving the entire left lung. Microscopic examination of biopsy sections disclosed very dense fibrous tissue containing multiple foci of granuloma and branching mycelia. Cultures grew an unknown species of Aspergillus.

The patient felt well until April, 1954. On July 4, 1954, he was admitted to the Scott and White Memorial Hospital. During these three months, he had developed a nonproductive cough, progressive dyspnea, nausea, anorexia, weakness, and hoarseness. He had lost 40 pounds in weight.

Physical examination: The patient was tall, pale, and thin. The left supraclavicular fossa contained a firm, round mass. Breathing was labored when the patient was in the sitting position, and became more difficult when he lay down. Breath sounds were decreased in intensity in the upper one-third of the posterior portion of the left lung, and were absent in the lower two-thirds. The right lung was normal. Over all valvular areas of the heart, a rough murmur was heard during systole. The patient's pulse was regular, 104 beats per minute.

Laboratory and roentgenographic findings: Complete blood counts were of essentially normal values except for one differential smear in which eosinophils numbered 23 per cent. *Aspergillus fumigatus* was cultured from the sputum. A roentgenogram of the chest (figure 1) revealed complete opacity of the left lung with displacement of the mediastinum to the right. The right lung appeared normal.

Treatment: The patient was given potassium iodide; isoproterenol (Isuprel®) by nebulization; aminophyllin, intravenously; corticotropin gel; sulfadiazine; streptomycin; positive-pressure oxygen therapy; and postural drainage. On this regimen, the patient's appetite increased, a sense of well-being developed, and ventilation of the left lung improved. He was dismissed from the hospital on August 17, 1954, with instructions to return in two weeks.

Second period of hospitalization: Because of pain in the right side of his chest, the patient was readmitted to the hospital after only five days. He had fever, 100°F., cyanosis of the lips and nail beds, pitting edema of the feet and legs, and orthopnea. Roentgenograms (figure 2) of the chest showed partial clearing of the left lung and shifting of the mediastinum back toward the midline. The electrocardiogram showed widespread changes in the T waves and S-T segments. Narcotics were required to relieve the pain. Failure of the right ventricle necessitated digitalization. Massive edema persisted. Although oxygen therapy was administered, the patient remained cyanotic. His urinary tract became obstructed. Horner's syndrome developed on his left side. On September 1, 1954, he became hypotensive, his pulse rate became rapid and irregular. Two hours later, he died.

Autopsy findings: Permission was granted for complete postmortem examination of the thoracic viscera and an examination *in situ* of the remaining viscera.

When the skin of the chest and abdomen was reflected, the subcutaneous tissue was found to be quite edematous. Because a dense fibrous process had extended from the rib cage into the skin, the skin of the thorax in the region of the left second, third, and fourth ribs was difficult to remove. Numerous enlarged lymph nodes were present in the left axilla. A well organized and partially canalized thrombus was found in the left subclavian vein.

The sternal plate was removed with difficulty. The upper two-thirds of its posterior surface was attached by dense fibrous tissue to a mediastinal mass. The structures of the mediastinum, including the base of the heart and the great vessels, were

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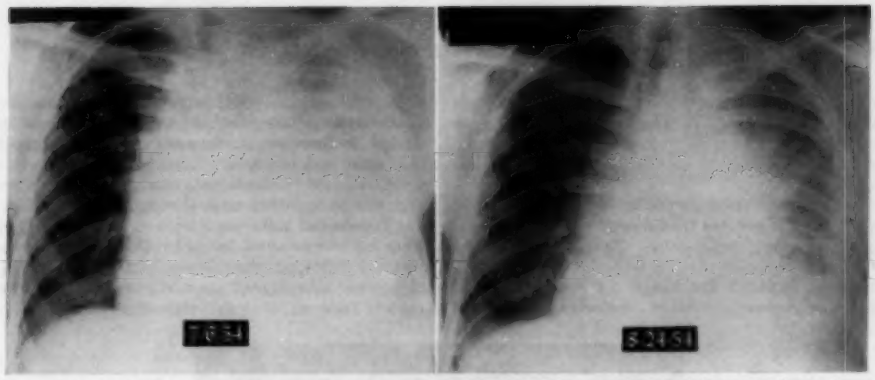

FIG. 1 (Left). Admission roentgenogram showing obliteration of the left lung field with displacement of the mediastinum to the right.

FIG. 2 (Right). Follow-up roentgenogram showing some clearing in the left lung.

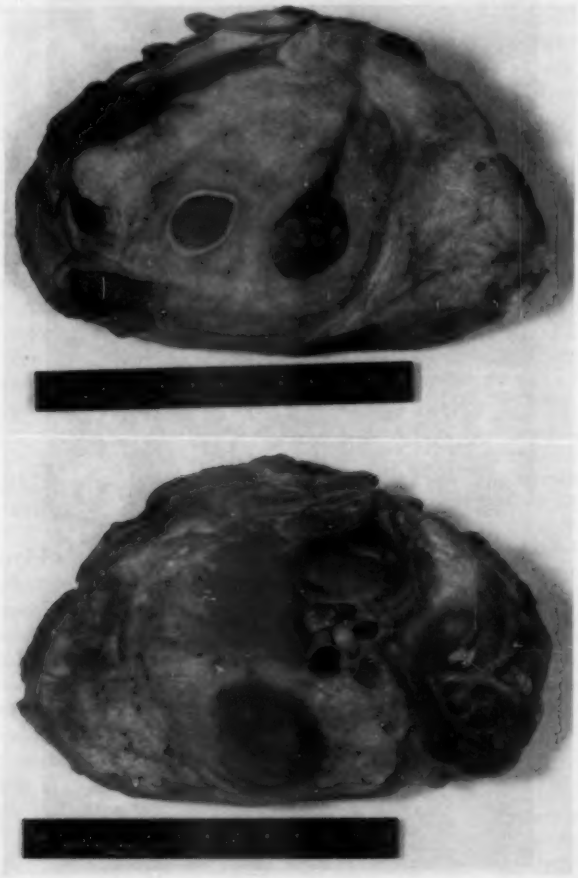

FIG. 3 (Upper). Autopsy specimen showing aspergillosis of the mediastinum with invasion of the vena cava.

FIG. 4 (Lower). Autopsy specimen demonstrating aspergillosis of the mediastinum with invasion of the myocardium.

incorporated in this mass, and the pericardial cavity was occluded by it. The portion of the mass which had extended into the neck could not be dissected free without mutilation of the neck and face.

The thoracic viscera were involved so markedly by the fibrous process that dissection of individual organs was not practicable. Transverse sections were made through various levels of the mediastinum and through the lungs. In several vessels, polypoid vegetations were projecting from the adjacent mass into the lumina; and the vena cava at its entrance into the right atrium was almost occluded by these vegetations (figure 3). The base of the heart was involved extensively, and little distinction could be made between the fibrous growth and the pericardium and the myocardium (figure 4). Pericardial adhesions were dense. Both lungs were infiltrated at all levels by the fibrous process, and the left lung was almost replaced by it. The right and left pleural cavities contained approximately 1,000 ml. of clear, yellow fluid.

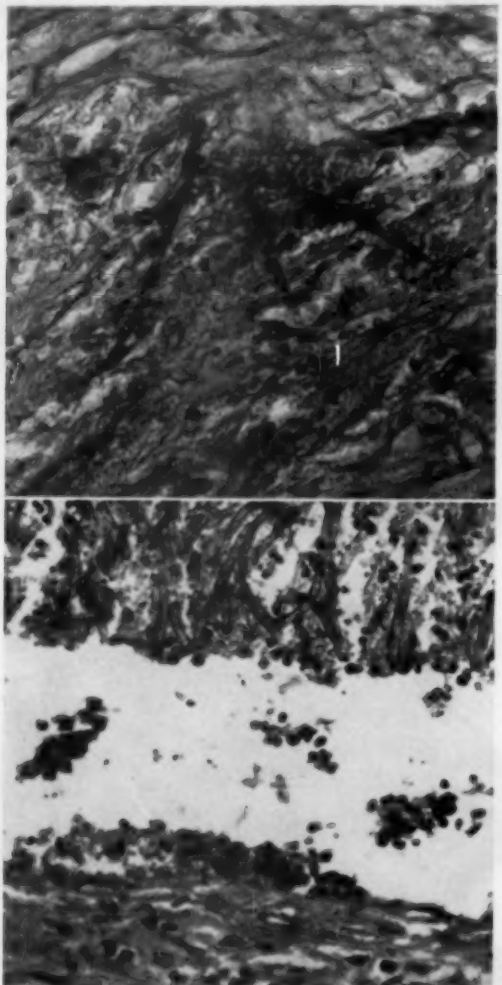

FIG. 5 (Upper). Photomicrograph demonstrating aspergillosis in the myocardium. (×250)
FIG. 6 (Lower). Photomicrograph of bronchial blood vessel with embolus of *Aspergillus fumigatus*.

Microscopically, a branching fungus with septate hyphae infiltrated all of the organs. A proliferative process characterized by various stages of fibrosis, ranging from young fibroblasts to mature fibrous tissue, was present. An occasional giant cell was found. The fungus and the associated proliferative process invaded the heart muscle (figure 5), the blood vessels, and the lung parenchyma. In some of the blood vessels and bronchi, solid colonies of fungus were observed, similar to those seen in the tissues (figure 6).

Discussion

Various classifications for pulmonary aspergillosis have been proposed, and several clinical types have been described (2–4). The severity ranges from mild tracheobronchitis to pulmonary infiltrates indistinguishable from bronchopneumonia, tuberculosis, neoplasm, and cavitary lesions. A localized bronchial lesion (mycetoma) which produces a characteristic picture on the roentgenogram has been demonstrated (5, 6). This has been called the "sign of the tegmentum" and is due to the growth of a "fungous ball" in a dilated bronchus topped by a thin, clear "tegmentum" or crescent of air in the unoccluded portion of the bronchial lumen. Parenchymal involvement is not apparent with this type of lesion.

Adequate criteria for the diagnosis of aspergillosis have not been established. Cawley (4) says that the diagnosis is based on repeatedly positive cultures, wherein the danger of contamination has been eliminated, together with finding the organism in biopsy specimens. According to Smith (1), the mycelial forms should be demonstrated in the discharges before the material is cultured.

In the case presented, the diagnosis of primary pulmonary aspergillosis was proved by positive cultures from biopsy and sputum specimens. Histologic examination further substantiated the diagnosis. A "frozen" mediastinum, such as might be expected in an invasive malignant process, and complete opacification of the left side of the chest with displacement of the mediastinum (demonstrated on roentgenograms) were the very unusual findings. These features of aspergillar infection have not been described previously.

The treatment of the more generalized forms of aspergillosis is unsatisfactory. Localized pulmonary or bronchial lesions can be excised (3, 7–9). When the drug first became available, Kelmenson (10) reported temporary closure of a bronchopleural fistula in one patient treated with amphotericin B. Recently, a report by Peer (11) indicates that the use of amphotericin B was "life-saving" in one patient with aspergillosis complicating pulmonary tuberculosis. Mycostatin (Nystatin®) has been used to eliminate the infection in the pneumonectomy space of two patients (12).

Summary

An unusual case of primary pulmonary aspergillosis with invasion and displacement of the mediastinum is reported. Roentgenograms showed complete opacity of the left side of the chest and shifting of the mediastinum. The diagnosis was proved by the demonstration of branching fungi with septate hyphae in biopsy specimens, as well as cultures of Aspergillus from the sputum. At autopsy, fibrous tissue proliferation was marked, and invasion by the fungus produced a frozen mediastinum which required that the entire thoracic contents be removed in a block. Branching fungi with septate hyphae were present in the tissues examined post mortem.

Sumario

Aspergilosis Pulmonar Primaria: Con Invasión del Contenido Mediastínico y de los Ganglios Linfáticos

Se presenta un caso extraño de aspergilosis pulmonar primaria con invasión y desplazamiento del mediastino. Las radiografías revelaron opacidad total del lado izquierdo del tórax y desviación del mediastino. Se confirmó el diagnóstico con el hallazgo de hongos ramificantes con hifas tabicadas en los ejemplares obtenidos por biopsia así como por cultivos del aspergilo en el esputo. En la autopsia, estaba acentuada la proliferación del tejido fibroso y la invasión por el hongo produjo un mediastino congelado que obligó a extraer en un bloque todo el contenido torácico. En los tejidos estudiados después de la muerte, había hongos ramificantes con hifas tabicadas.

Resume

Aspergillose pulmonaire primaire: avec envahissement du contenu médiastinal et des ganglions lymphatiques

Les auteurs rapportent un cas inhabituel d'aspergillose pulmonaire primaire avec envahissement et déplacement du médiastin. Les radio-

graphies montrèrent une opacité complète du coté gauche de la poitrine et un déplacement du médiastin. On prouva le diagnostic en démontrant les champignons à branches, avec les filaments divisés par un septum, dans les échantillons de biopsies, en même temps que dans des cultures d'aspergillus à partir de l'expectoration. A l'autopsie, la prolifération de tissu fibreux était marquée, et l'envahissement par le fungus produisit un médiastin bloqué qui força à enlever le contenu thoracique en bloc. Les champignons à branches, avec leurs filaments divisés par un septum, étaient présents dans les tissus examinés post-mortem.

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Notes

A NEW SYNTHETIC COMPOUND WITH ANTITUBERCULOUS ACTIVITY IN MICE: ETHAMBUTOL (DEXTRO-2,2′-(ETHYLENEDIIMINO)-DI-1-BUTANOL)

In the course of screening randomly selected synthetic compounds, N,N′-diisopropylethylenediamine was found to protect mice from an otherwise lethal infection with *Mycobacterium tuberculosis*, strain H37Rv. Following this lead, numerous related compounds were synthesized without appreciable improvement in activity until hydroxyl functions were introduced. The most promising compound of this series was found to be the dextro isomer of 2,2′-(ethylenediimino)-di-1-butanol dihydrochloride which has an efficacy index (tolerance/potency) similar to that of isoniazid when administered orally and superior to that of streptomycin when given parenterally. This substance is an odorless white crystalline compound; it is heat stable and highly soluble in water. A communication describing the synthesis and chemical characteristics of this compound is in press.[1]

The racemate of 2,2′-(ethylenediimino)-di-1-butanol dihydrochloride was evaluated in CF_1 adult female mice infected intravenously with the H37Rv strain. The compound was compared with isoniazid when administered orally (table 1) and with streptomycin when given parenterally (table 2). Treatments with graded doses of drugs, using 20 mice per treatment group, began on the day of infection and continued for fourteen consecutive days. Activity was based on the dose required to protect 50 per cent of the mice (ED_{50}) for sixty days after infection in tests in which all infected nontreated mice died within twenty-six days after infection. Evaluation data are summarized in table 3.

The compound was administered orally to mice infected with a bovine strain of *M. tuberculosis* resistant to the maximal tolerated dose of isoniazid, and subcutaneously to mice infected with a substrain of H37Rv fully resistant to streptomycin. These infections were lethal to 100 per cent (40/40) of the nontreated infected control mice within twenty days after infection. In each experiment, this compound was as effective against the resistant strains as against an H37Rv strain susceptible to both isoniazid and streptomycin.

The new compound was administered at various dosage levels with isoniazid as well as para-aminosalicylic acid in the drug diet, and was combined with streptomycin for subcutaneous administration to infected mice. No evidence of drug antagonism was observed.

The approximate median lethal single dose (LD_{50}) of the racemate of 2,2′-(ethylenediimino)-di-1-butanol dihydrochloride in noninfected adult mice was found to be greater than 12,800 mg. per kg. when administered orally, 1,600 mg. per kg. subcutaneously, between 800 and 1,600 mg. per kg. intraperitoneally, and between 200 and 400 mg. per kg. intravenously.

An agar diffusion microbiologic assay, based upon inhibition of *M. smegmatis* (ATCC 607), was developed and has been used to determine concentrations of ethambutol in serum and urine from mice, rats, dogs, and man. In mice receiving a single oral dose, a peak serum concentration was reached within one hour of ingestion. The compound was rapidly excreted. The peak concentration attained with either the maximal tolerated oral tubing or subcutaneous dose was about 100 γ per ml. The median effective dose (ED_{50}) by oral tubing or subcutaneous injection gave peak concentrations of about 3 γ per ml. and 7 γ per ml., respectively.

Studies *in vitro* have shown that concentrations of 1 to 4 γ of the racemate per ml. of liquid Sauton medium inhibited the growth of the H37Rv strain and isoniazid-resistant and streptomycin-resistant substrains. Similar concentrations inhibited the growth of the Brownell and Coffey strains[2] of Mycobacterium, as well as *M. smegmatis*, *M. ranei*, and *M. phlei*. The H37Rv strain has been transferred through eight consecutive passages in the presence of graded concentrations of the new compound. To date, the inhibitory concentration has not increased significantly. The compound was active only against organisms of the genus Mycobacterium. It was inactive *in vivo* and *in vitro* in tests with other bacteria, fungi, and viruses.

Recent testing *in vitro* and *in vivo* of the stereoisomers of 2,2′-(ethylenediimino)-di-1-butanol has shown that the activity can be accounted for

[1] Wilkinson, R. G., *et al.*: J Amer Chem Soc (in press).

[2] The Coffey and Brownell strains, as well as the isoniazid-resistant and streptomycin-resistant strains of *M. tuberculosis*, were obtained through the courtesy of Dr. William Steenken, Jr., Director of Trudeau Laboratory, Saranac Lake, New York.

TABLE 1

ORAL EFFICACY OF THE RACEMATE OF 2,2′-(ETHYLENEDIIMINO)-DI-1-BUTANOL DIHYDROCHLORIDE AND ISONIAZID IN TUBERCULOUS MICE

Daily	Alive/Total (Per Cent Survival) on 60th Day post Infection				Daily				
	Oral Tubing Treatment					Drug Diet Treatment			
	Racemate*		Isoniazid			Racemate		Isoniazid	
mg./kg.					*mg./kg.*				
6,400	18/20	(90)†			4,800	10/10	(100)		
3,200	20/20	(100)			2,400	20/20	(100)		
1,600	19/20	(95)			1,200	20/20	(100)		
800	20/20	(100)			600	20/20	(100)	Toxic	
400	20/20	(100)			300	20/20	(100)	10/10	(100)
200	20/20	(100)	Toxic		150	17/20	(85)	10/10	(100)
100	14/20	(70)	18/20	(90)	75	5/20	(25)	8/10	(80)
50	0/20	(0)	16/20	(80)	38	1/20	(5)	20/20	(100)
25			20/20	(100)	19			18/20	(90)
12			16/20	(80)	10			20/20	(100)
6			20/20	(100)	5			19/20	(95)
3			17/20	(85)	3			0/20	(0)
1.5			16/20	(80)					
0.8			3/20	(15)					

80/80 infected control mice died between the fourteenth and twenty-sixth days after infection with a mean survival time of seventeen days.

* Racemate consists of 50 per cent ethambutol and 50 per cent inactive levo isomer.

† Figures in parentheses indicate per cent survival.

TABLE 2

PARENTERAL EFFICACY OF THE RACEMATE OF 2,2′-(ETHYLENEDIIMINO)-DI-1-BUTANOL DIHYDROCHLORIDE AND STREPTOMYCIN IN TUBERCULOUS MICE

Dose	Alive/Total (Per Cent Survival) on 60th Day post Infection							
	Subcutaneous Treatment				Intraperitoneal Treatment			
	Racemate*		Streptomycin		Racemate		Streptomycin	
mg./kg./day								
1,600	Toxic				Toxic			
800	20/20	(100)†	Toxic		18/20	(90)	Toxic	
400	18/20	(90)	19/20	(95)	20/20	(100)	18/20	(90)
200	19/20	(95)	15/20	(75)	20/20	(100)	17/20	(85)
100	12/20	(60)	10/20	(50)	16/20	(80)	9/20	(45)
50	3/20	(15)	8/20	(40)	11/20	(55)	4/20	(20)
25	3/20	(15)	4/20	(20)	3/20	(15)	0/20	(0)
12	0/20	(0)	0/20	(0)	0/20	(0)		

80/80 infected control mice died between the fourteenth and twenty-sixth days after infection with mean survival time of seventeen days.

* Racemate consists of 50 per cent ethambutol and 50 per cent inactive levo isomer.

† Figures in parentheses indicate per cent survival.

on the basis of the *dextro* isomer (ethambutol)[3] alone. The *dextro*, *levo*, and *meso* isomers are equally toxic to mice. As shown in table 4, the *levo* isomer is inactive at the maximal tolerated dose and the *meso* isomer is only one-sixteenth as active as the *dextro* form.

[3] Ethambutol is the generic name reserved for the dextrorotatory form of 2,2′-(ethylenediimino)-di-1-butanol by Lederle Laboratories Division of the American Cyanamid Company.

In summary: Dextro-2,2′-(ethylenediimino)-di-1-butanol is a new compound with activity *in*

TABLE 3

TOLERANCE AND POTENCY OF THE RACEMATE OF 2,2′-(ETHYLENEDIIMINO)-DI-1-BUTANOL DIHYDROCHLORIDE, ISONIAZID, AND STREPTOMYCIN IN TUBERCULOUS MICE

Dose	Oral Treatments				Parenteral Treatments			
	Oral Tubing		Drug Diet		Subcutaneous		Intraperitoneal	
	Racemate*	Isoniazid	Racemate	Isoniazid	Racemate	Streptomycin	Racemate	Streptomycin
mg./kg./day								
Maximal tolerated dose (MTD)	6,400	100	4,800	300	800	400	800	400
Median effective dose (ED_{50})	90	1.2	94	3.2	90	80	50	100
Efficacy index (MTD/ED_{50})	70	80	50	94	9	5	16	4

* Racemate consists of 50 per cent ethambutol and 50 per cent inactive levo isomer.

TABLE 4

EFFICACY OF THE STEREOISOMERS OF 2,2′ (ETHYLENEDIIMINO)-DI-1-BUTANOL DIHYDROCHLORIDE IN TUBERCULOUS MICE

Subcutaneous Dose	Alive/Total (Per Cent Survival) on 30th Day post Infection			
	Stereoisomers			
	Racemate	Dextro*	Levo	Meso
mg./kg./day				
1,600	Toxic	Toxic	Toxic	Toxic
800	10/10 (100)†	5/5 (100)	0/10 (0)	5/5 (100)
400	10/10 (100)	5/5 (100)	0/10 (0)	4/5 (80)
200	10/10 (100)	10/10 (100)	0/5 (0)	1/5 (20)
100	10/10 (100)	10/10 (100)	0/5 (0)	0/5 (0)
50	7/10 (70)	10/10 (100)		0/5 (0)
25	1/10 (10)	7/10 (70)		
12	1/10 (10)	2/10 (20)		
6	0/10 (0)	0/10 (0)		

20/20 infected control mice died between the twelfth and twenty-second days after infection with mean survival time of seventeen days.

* Ethambutol[3]

† Figures in parentheses indicate per cent survival.

vivo and *in vitro* against mycobacteria. It is active orally as well as parenterally, and it is active against strains of *M. tuberculosis* which are resistant to isoniazid and streptomycin. On an oral dosage basis, the efficacy (tolerance/potency) index of the new compound is similar to that of isoniazid; parenterally it is superior to streptomycin. The new drug is compatible in combinations with isoniazid, as well as with streptomycin.

Reports covering various phases of this work are in preparation.

[4] The writers acknowledge the valuable assistance of Dr. H. J. White and Dr. S. Kushner.

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DEATHS AND ILLNESS FROM BRONCHITIS, EMPHYSEMA, AND ASTHMA

A good deal has been written about chronic bronchitis recently, especially in England, where bronchitis (or its complications) is a major cause of death and is one of the most important causes of incapacity for work. Use of bronchitis death rates for the purpose of comparing respiratory disease patterns of various countries is apt to lead to the erroneous impression that bronchitis-like conditions cause few deaths in the United States.

The purpose of this Note is twofold: first, to call attention to differences in terminology relating to bronchitis, pulmonary emphysema, and asthma, and to the problem of interpretation of certain vital statistics that is created by these differences; second, to examine the mortality rates for these diseases in the United States and to raise some questions about recent changes in these rates.

The meaning of "bronchitis" deaths: Bronchitis death rates are difficult to interpret for several reasons: (*1*) the category *bronchitis* in the International List of Causes of Death includes chronic bronchitis and acute bronchitis, two distinct entities; (*2*) certain terms (bronchitis among them) are more popular in some countries than in others; (*3*) definitive criteria for the clinical diagnosis of many diseases, including bronchitis, do not exist or are not uniform; and (*4*) the means of determining and listing the cause of death may vary from one country to another.

The figures in table 1, therefore, serve only as a crude guide for estimating deaths from a group of ill-defined chronic respiratory ailments which may or may not be called bronchitis. This group includes chronic bronchitis, pulmonary emphysema, asthma, bronchiectasis, and other conditions associated with pulmonary fibrosis. All share certain of the following characteristics: chronic or recurrent cough, expectoration, abnormal breathlessness, wheezing, recurrent pulmonary infections, and cardiac failure.

Present concepts of bronchitis, emphysema, and asthma: Understanding of the epidemiology and pathogenesis of these diseases suffers because of a lack of uniform terminology which, as Fletcher has pointed out, stems chiefly from the subjective nature of many of the diagnostic criteria used in clinical medicine.[1]

The term chronic bronchitis has different connotations in various countries. In the United Kingdom it is a confidently diagnosed affection of the bronchi and bronchioles, with cough and expectoration as its outstanding symptoms, and emphysema, bronchopneumonia, and cor pulmonale its chief complications.[2,3] Chronic bronchitis by itself is rarely, if ever, a cause of death. In the past some have preferred to diagnose the condition only in the presence of undue breathlessness[2,3]; the opinion of many interested in epidemiologic aspects of the disease is that the presence of breathlessness is not required for the diagnosis.[4-6] Chronic bronchitis is defined as "a condition of subjects with chronic or recurrent excessive mucous secretion in the bronchial tree," the diagnostic criterion being chronic or recurrent cough with expectoration, not solely attributable to other conditions of the lungs.[6] The virtue of such a definition is that it focuses attention on early phases of the disease rather than on its complications.

Chronic bronchitis, while receiving scant attention in American medical literature, is by no means uncommon in this country.[7,8] In 1940, an estimated 1,700,000 persons were said to be subject to the condition.[9] Adequate descriptions of the disease have made every physician familiar with the term, yet at the present time it is not a popular one and is looked upon as somewhat of a "waste-basket diagnosis." Consequently, it often goes by other names, such as asthma, asthmatic bronchitis, emphysema, bronchiectasis, or "smoker's cough."

What American physicians generally call emphysema (or sometimes asthma) is closely akin to the syndrome referred to as "bronchitis with emphysema" or simply "chronic bronchitis" in the United Kingdom. Although the relationship between chronic bronchitis and subsequent emphy-

[1] Fletcher, C. M.: Amer Rev Resp Dis, 1959, *80*, 483.

[2] Stuart-Harris, C. H., and Hanley, T.: Chronic Bronchitis, Emphysema and Cor Pulmonale, John Wright and Sons Ltd., Bristol, 1957.

[3] Recent Trends in Chronic Bronchitis, edited by N. C. Oswald, Lloyd-Luke Ltd., London, 1958.

[4] Ogilvie, A. G., and Newell, D. J.: Chronic Bronchitis in Newcastle-upon-Tyne, E. and S. Livingstone Ltd., Edinburgh, 1957.

[5] Fletcher, C. M., *et al.*: Brit Med J, 1959, *2*, 257.

[6] Fletcher, C. M., *et al.*: Thorax, 1959, *14*, 286.

[7] Phillips, R. W., *et al.*: Dis Chest, 1954, *26*, 520.

[8] Pemberton, J.: AMA Arch Industr Health, 1956, *13*, 529.

[9] Britten, R. H., *et al.*: Public Health Rep, 1940, *55*, 444.

sema is accepted by many,[2-9,7,10] it is by no means an invariable one.[11] Not infrequently, persons with well-established emphysema present with dyspnea alone, later developing cough with expectoration, indicating that emphysema is not necessarily preceded by overt bronchitis.[10,12-14] Primary bronchiolar or alveolar involvement may be important in these cases.

Comparison of death rates helps point out the apparent difference in thinking between Americans and British on the subject of factors predisposing to emphysema. In the United States, for example, the ratio of deaths from

"chronic bronchitis with emphysema" (502.0)[15]
"emphysema without mention of bronchitis" (527.1)

is about 1 to 6, whereas for England and Wales this ratio is about 10 to 1.[16] In other words, vital statistics suggest that the British nearly always associate pulmonary emphysema with preceding or concomitant chronic bronchitis, whereas Americans rarely appear to. The explanation for this may be either that emphysema as seen in the United States is truly "idiopathic" and not preceded by bronchitis or that the American concept of emphysema includes within its framework what the British call bronchitis. It might be argued that the latter is really emphysema in the sense of a pathologic lesion, and, indeed, long-standing bronchitis almost invariably shows some associated emphysema.[2] Differentiation between bronchitis and emphysema at the clinical and physiologic level is often difficult and, of the two diseases, bronchitis can be diagnosed with much more certainty.

In both countries the term emphysema is being used more often in recent years. In England and Wales the ratio (among men) of deaths from

"chronic bronchitis with emphysema" (502.0)
"other chronic bronchitis" (502.1)

TABLE 1

ACUTE, CHRONIC, AND UNQUALIFIED BRONCHITIS DEATH RATE PER 100,000, 1955

England and Wales	64.8	Holland	11.5
Scotland	40.6	Australia	9.9
Italy	27.0	France	4.1
Belgium	21.6	Norway	3.7
New Zealand	20.3	Canada	3.5
West Germany	13.1	United States	1.9

Source: W.H.O. Epidemiological and Vital Statistics Report. 1958.

has gone from about 1 to 2 to 1 to 1 in the past decade as a result of an increase in deaths attributed to "chronic bronchitis with emphysema." Likewise, the death rate for "emphysema without mention of bronchitis" has nearly doubled during the past ten years.[17] These latter figures might suggest that more cases of "idiopathic" emphysema are being seen in England recently.

The slight attention paid to chronic bronchitis in this country as compared with that in England undoubtedly reflects quantitative differences in the disease on the two sides of the Atlantic—differences which may bear some relationship to climate, air pollution, urbanization, and smoking habits in the two countries.

Emphysema: Clinicians, physiologists, radiologists, and pathologists are apt to define emphysema according to their own disciplines. The requisite symptom for the clinical diagnosis is exertional breathlessness not due to other heart or lung diseases; but this suffers from its lack of objectiveness. The presence of emphysema (anatomic) can often be asserted with confidence on clinical grounds alone, but the correlation between clinical and physiologic data, on the one hand, and lung pathology, on the other, may be surprisingly poor.[1,6] In established cases the principal physiologic abnormalities include evidence of slowing of expiratory airflow, pulmonary hyperinflation, impaired diffusing capacity for oxygen and carbon monoxide, and uneven ventilation-blood-flow relationship. Many agree that the term *pulmonary emphysema* would best be reserved for the description of a pathologic condition. At the 1958 Ciba Guest Symposium, emphysema was defined as being characterized by "an increase beyond the normal in the size of air spaces distal to the terminal bronchiole, either from dilatation or from destruction of their walls"[6]; techniques that fix the lung in a state of physiologic distention are necessary for proper study of the lungs.

A proposal has been made to substitute the

[10] GAENSLER, E. A., AND LINDGREN, I.: Amer Rev Resp Dis, 1959, *80* (Supplement, p. 185).

[11] MAYER, E., AND RAPPAPORT, I.: JAMA, 1957, *165*, 1227.

[12] DOBELL, H.: On Winter Cough, Catarrh, Bronchitis, Emphysema and Asthma, J. and A. Churchill, London, 1875.

[13] FOWLER, J. K., AND GODLEE, R. J.: The Diseases of the Lungs, Longmans Green and Co., London, 1898.

[14] FITTS, C. H.: Postgrad Med J, 1958, *34*, 184.

[15] Numbers in parentheses are the classification numbers of the Seventh Revision of the International List of Causes of Death, 1958.

[16] United States figures from the National Office of Vital Statistics. Figures for England and Wales from the General Register Office.

[17] GENERAL REGISTER OFFICE: Personal communication.

phrase "irreversible obstructive lung disease" for the term emphysema in clinical use, reserving emphysema for the lesion described by the pathologist. The word emphysema is so thoroughly a part of clinical medicine, however, that the success of such a proposal is to be doubted. If the clinician would emphasize that he uses the term emphysema to describe a certain clinico-physiologic syndrome, confusion would be avoided to an extent.

Asthma: The term "asthma" has been used through the ages to describe various conditions and still is more descriptive than definitive. Bronchial asthma may occur at any age and refers to the "condition of subjects with widespread narrowing of the bronchial airways which changes its severity over short periods of time and is not due to cardiovascular disease."[9] The clinical features are episodic wheezing, dyspnea, cough and expectoration, and demonstrable evidence of reversible airway obstruction and, at times, of allergy. Emphysema may be a complication. It is apparent that a number of chronic respiratory conditions can simulate asthma. For example, the very high death rate for asthma in Japan (15.6 per 100,000 in 1957, a rate nearly four times that for the United States and England) undoubtedly is a reflection of the fact that many conditions, among them tuberculosis, bronchitis, bronchiectasis, emphysema, and cardiac diseases, are likely to be diagnosed as asthma, especially in older persons.[10] In the United States the peak asthma case rate in adulthood is around age sixty-five,[10] and the age-specific death-rate pattern for asthma is essentially the same as for emphysema.[10] These figures support the view that the term asthma is often used interchangeably with emphysema and/or chronic bronchitis. "Intermittent or reversible obstructive lung disease" has been suggested as a phrase descriptive of what is presently called asthma.[6]

Bronchiectasis and pulmonary fibrosis: Deaths from bronchiectasis (526)[10] and chronic interstitial pneumonia (525) occur chiefly in men past middle age. Symptoms of either condition may be similar to those of asthma, bronchitis, or emphysema. Bronchiectasis denotes a condition of dilation of bronchi demonstrable by the radiologist or pathologist; chronic interstitial pneumonia, a diagnosis made by the pathologist, refers to a variety of conditions such as pulmonary fibrosis or chronic inflammatory processes, any of which may precede, accompany, or follow the development of bronchitis and/or emphysema.

[18] Japanese Ministry of Health and Welfare: Personal communication.

[19] Collins, S. D., *et al.*: Public Health Monograph No. 25, Public Health Service Publication No. 390, 1955.

[20] Bower, G.: Unpublished data.

Morbidity of bronchitis, emphysema, and asthma: A number of epidemiologic studies in England have established the impressive incidence of chronic bronchitis, especially among industrial and laboring populations and in men past middle age.[2,4,5,21,22] Studies of this sort are virtually nonexistent in this country.[7,8]

In a review of six American health surveys (1928 to 1943), the most common illnesses were respiratory: coryza, bronchitis, and influenza.[10] The annual case rate for bronchitis was 103 per 1,000 population, the annual disability[23] case rate was 51 per 1,000, and the average duration of disability seven and one-half days per case. In a sizeable fraction of cases a physician was not in attendance, casting doubt on the validity of the diagnosis. In a recent general practice study in England, the annual "patients' consulting-rate" for bronchitis was 61 per 1,000 population.[24] Bronchitis case rates in the United States do not show the striking predominance in elderly men seen in Great Britain. Moreover, the duration of incapacity from spells of bronchitis in Great Britain (25 per cent of incapacitating spells in 1951 lasted more than twenty-four days) weighs heavily in favor of the view that British bronchitis, alone or with its complications, is a more serious disease than that in the United States or that the term is used in this country to describe a benign condition such as a "cold" with cough and absence of a day or two from work, and one occurring in fewer persons already subject to chronic bronchopulmonary disease.

The number of persons totally disabled by bronchitis and emphysema in the United States is not known. In the National Health Survey of 1937 it was found that 42 persons per 100,000 population had been disabled for the preceding twelve months by asthma or other chronic nontuberculous respiratory disease. This represented about 4 per cent of all disabilities recorded.[9] Data for 1957 relating to recipients of Aid to the Permanently and Totally Disabled (a rapidly expanding federal-state program with more than 300,000 persons, chiefly between the ages of fifty and sixty-four, who received aid in 1958) indicate that the primary diagnosis was emphysema in 8 per cent and bronchitis in

[21] Oswald, N. C., and Medvei, V. C.: Lancet, 1953, *2*, 843.

[22] Higgins, I. T. T., *et al.*: Brit Med J, 1956, *2*, 904.

[23] A disabling illness was one which prevented a person from carrying out his or her usual job or household duties for at least one day.

[24] Logan, W. P. D., and Cushion, A. A.: Morbidity Statistics from General Practice: Studies on Medical and Population Subjects No. 14, General Register Office, London, H.M.S.O., 1958.

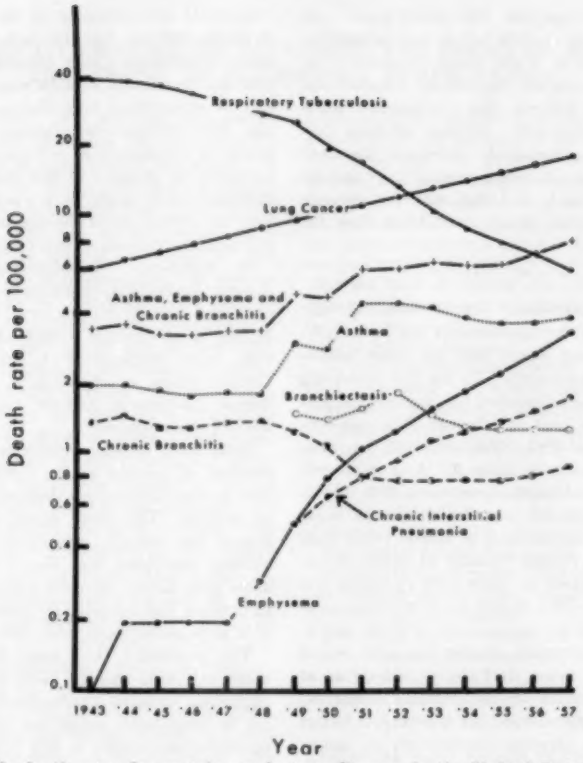

Fig. 1. Crude death rates for certain respiratory diseases in the United States, 1943–57.
Sources: Vital Statistics of the United States.

0.8 per cent.[25] Among men, emphysema ranked second to arteriosclerotic heart disease as a cause of disability.

Estimates of the prevalence of asthma in the general population range up to 3 or 4 per cent.[26] In the National Health Survey of 1937 an estimated 3,450,000 persons were said to have asthma.[9]

Recent trends in the United States: Within the past fifteen years "emphysema without mention of bronchitis" (527.1)[13] has shown a remarkable increase as a listed cause of death in this country. The trend is shown in figure 1; at the present time emphysema accounts for about as many deaths as does pulmonary tuberculosis. Of further interest is the fact that emphysema is often listed on death certificates as a contributing but not immediate cause of death. The death rate for emphysema as well as asthma showed only small absolute changes between 1900 and 1940; the rate

TABLE 2

AGE-SPECIFIC DEATH RATES FOR EMPHYSEMA, CHRONIC BRONCHITIS, AND ASTHMA COMBINED

Age	Deaths per 100,000		
	1945	1950	1955
35–9	1.3	1.3	1.4
40–4	2.3	2.4	2.6
45–9	3.5	3.9	4.3
50–4	5.6	6.6	7.8
55–9	8.1	10.6	15.3
60–4	9.5	15.5	24.3
65–9	11.7	18.4	34.4
70–4	15.3	23.2	40.4
More than 75	28.5	33.4	54.3

[25] Selected data on characteristics of applicants under the disability provisions of the old age, survivors, and disability insurance program, January–December, 1957, U. S. Department of Health, Education, and Welfare, 1958.

[26] VAUGHAN, W. T.: Practice of Allergy, C. V. Mosby Co., St. Louis, 1954.

for chronic bronchitis, on the other hand, fell steadily. The cause of this fall is not altogether apparent. Changes in crude death rates between 1943 and 1957 for certain respiratory diseases can be summed up as follows: the tuberculosis death rate dropped spectacularly; the rate for lung cancer (primary and unspecified) increased; the rate for chronic bronchitis, emphysema, and asthma (combined) increased, and the rate for chronic interstitial pneumonia nearly paralleled that for emphysema.

* * *

For practical purposes, deaths from chronic bronchitis, emphysema, and asthma can be lumped together. The rising death rate for these conditions combined is accounted for by the increasing numbers of deaths attributed to emphysema and asthma in persons past middle age. The age-specific death rates in 1945, 1950, and 1955 for these conditions are shown in table 2.[27] A possible explanation for the changes observed is that physicians have more readily certified deaths as being from emphysema or asthma in recent years than in previous years, either because of more awareness of these diseases or more care in filling out death certificates. The factor of better diagnosis would not appear to account for a very significant part of the increase during the past fifteen years, one reason being that the person dying of emphysema or asthma usually exhibits unmistakable evidence of the disease. At any rate, if better diagnosis or more accurate certification are major factors in the changing death rate, the actual death rate for these diseases is probably even higher than present figures indicate. On the other hand, if the rising death rate for chronic bronchitis, emphysema, and asthma combined is genuine, potent environmental factors must surely be involved in the genesis of such a change.

[27] Sources: Vital Statistics of the United States, 1945; data from individual state departments of public health, 1950 and 1955; population estimates, U. S. Department of Commerce.

Accurate interpretation of the changes described is made difficult by the fact that the diseases under consideration have different meanings to various people. Available data suggest that the problem of obstructive lung disease is steadily growing. Standardized terminology relating to this group of conditions is needed. Meaningful interpretation of changes in the death rates for these diseases can be made only when there is certainty that the definitions of the diseases are standardized and remain constant over a period of time.

In summary: Standardized definitions for chronic bronchitis, pulmonary emphysema, and bronchial asthma do not exist. Emphasis in Great Britain is on chronic bronchitis as the initial disease, with emphysema a complication. In the United States, emphysema is often considered a disease *sui generis*. Asthma is a term applied to a variety of conditions.

Data from certain studies indicate that a large number of persons in the United States are subject to obstructive lung diseases of various degrees of severity. The crude death rate in the United States for chronic bronchitis, emphysema, and asthma combined has shown a steady increase since 1948. Death rates for emphysema and asthma in persons past middle age have shown a considerable increase between 1945, 1950, and 1955.

The possibility that these diseases are causing more and more deaths (and disability) makes it important to have standardized terminology relating to them, and points out the need for further epidemiologic studies in this field.

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INEFFECTIVENESS OF NICOTINAMIDE AND ISONIAZID IN THE TREATMENT OF PULMONARY TUBERCULOSIS[1]

Previously published data derived from extensive animal work have indicated that nicotinamide, a compound which is very closely related to the isoniazid-pyrazinamide group, has definite antituberculous activity. Chorine[2] first demonstrated this in 1945, and since that time several reports have indicated the possible clinical usefulness of this compound. McKenzie and associates,[3] in screening a large number of compounds for antituberculous activity, concluded that nicotinic acid and its amide were quite active and, in very large doses, were found to suppress the spread of tuberculosis in experimentally infected mice. Also, in a brief report from Fust and Studer,[4] nicotinamide was found to exceed PAS, a thiosemicarbazone, and even streptomycin in antituberculous effectiveness in mice. In the writers' laboratory,[5] it was demonstrated that nicotinamide was definitely active against strain H37Rv, *in vitro*, although less so than pyrazinamide. Moreover, in further studies in mice using the microbial enumeration technique, it was found that nicotinamide alone had an antituberculous effect that was comparable to that of streptomycin alone. Of even greater interest was the finding that nicotinamide antagonized the action of isoniazid in the early days of the experimental tuberculous infection, as did pyrazinamide.

Obviously, it could not be determined *a priori* whether, in a *clinical* situation, nicotinamide plus isoniazid would follow the pattern seen in mice or, if it would (as does pyrazinamide) evidence initial isoniazid antagonism followed by enhancement of antituberculous effectiveness. Accordingly, a clinical trial of nicotinamide in conjunction with isoniazid was undertaken and is reported herewith.

Previously untreated patients with either moderately advanced or far advanced, uncomplicated, pulmonary tuberculosis were admitted to the study group. Of the 48 patients in the study, 32 were admitted to The Fort Defiance Indian Hospital and 16 to The New York Hospital-Cornell Medical Center. Fifteen of the original 48 patients were eliminated from the series before completion of the study: 4 patients who left the hospital against medical advice, 2 patients because it was decided that the type of disease did not meet the predetermined criteria, and 9 patients because they had been in the study less than three months at the time of final evaluation.

In the final combined series of 33 patients, 10 were classified roentgenographically as having moderately advanced, and 23, far advanced disease. Cavitation was present at the start of therapy in 32 of these 33 patients, and the diagnosis was confirmed by bacteriologic studies.

The chemotherapy regimen employed consisted of 5 mg. of isoniazid per kg. per day and 50 mg. of nicotinamide per kg. per day.

The course of the disease was followed by daily clinical evaluation, monthly chest roentgenograms, and monthly sputum and/or gastric aspirations for culture of tubercle bacilli.

The 33 patients available for final evaluation all received isoniazid-nicotinamide therapy for at least three months, and 15 patients received this drug pairing for a total of six months.

The following results were recorded:

Following three months of therapy, 11 patients (of the 33) had shown substantial roentgenographic improvement; 10 had shown slight improvement; 8 no improvement; and the disease had definitely deteriorated in 4 patients. Three of the 4 patients whose disease showed progression at the three-month point regained at least their pretreatment status in the ensuing period; but, in one patient, it was necessary to change to conventional therapy because of considerable progression of disease at the end of six weeks.

Ten of the 15 patients treated for a six-month period showed substantial roentgenographic improvement; 3 showed slight improvement; and 2, no improvement.

Cavity closure was achieved in 6 of the 32 patients in whom cavities were originally demonstrated. This closure occurred at the three-month point in 3 patients and at the six-month point in 3 patients.

The sputum of 9 of the 33 patients in this series became negative for *M. tuberculosis*. Reversal of infectiousness had occurred by the three-month point in 4 patients and by the six-month point in 5 patients.

[1] This study was supported in part by grants from the Navajo Tribal Council; the Committee on Medical Research of the American Thoracic (formerly Trudeau) Society, medical section of the National Tuberculosis Association; and Research Grant E-635 from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service.

[2] Chorine, V.: C R Acad Sci (Par) 1945, *220*, 150.

[3] McKenzie, B. S., *et al.*: J Lab Clin Med, 1948, *33*, 1249.

[4] Fust, B., and Studer, A.: Helv Med Acta, 1951, *18*, 449.

[5] McDermott, W., and Tompsett, R.: Amer Rev Tuberc, 1954, *70*, 746.

For the most part, the clinical course was unremarkable. One patient developed drug fever, which was attributed to isoniazid. This occurred very early in the course of therapy, and the patient has not been included in the analysis since he received the drug pairing for a very short period of time. As mentioned previously, in a second patient the disease showed considerable progression at the end of six weeks of therapy, requiring change to conventional therapy.

Toxicity attributable to nicotinamide was not observed in any of the patients treated.

* * *

During the course of this study it became increasingly apparent that this drug pairing, nicotinamide-isoniazid, in the dosage employed did not produce satisfactory results. After sufficient time had elapsed to be sure that the poor results were not attributable to other causes, the treatment program was stopped. The antagonism of isoniazid by either pyrazinamide or nicotinamide observed in the mice[6] apparently has significance in man in the case of nicotinamide.

In only 8 of the original 33 patients was a favorable result obtained, i.e., patients whose treatment could be completed on isoniazid alone on an outpatient basis after cessation of the nicotinamide. The remaining 25 patients, 8 of whom were classified as having moderately advanced and 17 as having far advanced disease, required therapy in addition to isoniazid after stopping nicotinamide. Additional therapy was required in 22 of these patients because of persistence of open cavities with infectious sputum, in 2 because of the "open-negative" syndrome, and in one because of marked deterioration of the disease. This is in marked contrast to the results achieved by the present writers in similar clinical trials with isoniazid alone[7] wherein 78 per cent of the patients showed substantial improvement.

It is apparent that the combination of isoniazid with nicotinamide was distinctly inferior in comparison with isoniazid alone, isoniazid-pyrazinamide, or any of the commonly employed multiple-drug regimens. Moreover, the ability to inhibit isoniazid early in the treatment period, which is demonstrated by both nicotinamide and pyrazinamide in the experimental model, appears to be much more complete and persistent in the case of nicotinamide. However, because of the inherent limitations of any clinical trial, it was not appropriate to continue the study in order to evaluate the degree of persistence of the nicotinamide inhibition of isoniazid.

In summary: A group of 33 previously untreated patients with moderately and far advanced pulmonary tuberculosis were treated with nicotinamide and isoniazid for a minimal period of three months. After three months of therapy only 11 patients had shown substantial roentgenographic improvement and 4 had already shown clinical deterioration. At the six-month period, reversal of infectiousness had occurred in only 9 of the 33 patients who received nicotinamide with isoniazid, compared with a 72 per cent incidence of reversal of infectiousness in a previous clinical trial with isoniazid alone. The total clinical results with isoniazid-nicotinamide were so poor that the study was terminated in the interest of the patients, and appropriate antituberculous drugs were added to isoniazid as indicated for each individual patient. It was concluded that, in the dosages used in this study, isoniazid and nicotinamide were contraindicated as an antituberculous chemotherapy.

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[6] McCune, R. M., *et al.*: J Exp Med, 1956, *104*, 763.

[7] Jordahl, C. W., *et al.*: Amer Rev Tuberc, 1958, 77, 539.

MYCOBACTERIOPHAGE LYSATES AS SEROLOGIC ANTIGENS

A wide variety of antigens has been employed in serologic tests for tuberculosis. These have included killed whole tubercle bacilli,[1,2] Old Tuberculin,[3,4] aqueous or solvent extracts of bacilli,[5,6] fractions of tubercle bacilli obtained by chemical fractionation,[1,7] and concentrated culture filtrates.[8,9] The majority of these methods seem sufficiently drastic to destroy or modify antigenic activity.[10]

The availability of mycobacteriophages capable of rapidly lysing masses of mammalian tubercle bacilli and other acid-fast mycobacteria[11,12] suggested a study of the use of such lysates as serologic antigens. This Note describes the results of a study comparing a phage lysate antigen (D29 antigen) with the culture filtrate antigen of Parlett and Youmans,[13] as employed in the gel double-diffusion test currently under study in this laboratory.

The procedure reported is a modification of a technique described elsewhere.[11] Approximately 2.0 ml. of a 10-day-old Dubos Tween®-albumin fluid culture of *Mycobacterium tuberculosis*, strain H37Rv (OVH #797), and 2.0 ml. of phage D29 (approximate titer, 10^9 particles per ml.) were pipetted into 180- to 240-ml. plastic screw-capped prescription bottles which contained a basal layer of 40 ml. of Bordet-Gengou agar base on the flat side of the bottle. The Bordet-Gengou agar base was supplemented with 0.5 per cent yeast extract and 1.0 per cent tryptone. The bottles were incubated for fifteen minutes to allow for the adsorption of the phage to bacterial cells, after which 4.5 ml. of semi-solid Bordet-Gengou agar, melted and cooled to approximately 50°C., were added to each bottle, quickly mixed with the phage-cell inoculum, and the bottle was placed on its side, allowing the semi-solid mixture to layer over the basal agar and solidify. A control containing bacterial cells without phage was prepared similarly.

All bottles were incubated at 37°C. for five days, at which time the control showed growth, whereas the bottles containing phage were clear, indicating lysis of the inoculum. The phage lysate was harvested by adding 10 ml. of heart infusion broth supplemented with calcium ion (0.002 M per ml.) to each bottle after first macerating the thin-solid agar layer with a sterile glass rod. Thirty minutes later, the fluid from each bottle was coarse-filtered through Whatman #43 filter paper, and then filtered through a Seitz filter. Lysates prepared in this manner were used in these experiments without further concentration.

Dated Parlett-Youmans antigen was furnished by Parlett, and this concentrated antigen was used according to his directions.

Serum specimens: One hundred and three serum specimens from patients newly admitted to the Olive View Hospital with a provisional diagnosis of tuberculosis were employed. These specimens from fasting and nonfasting patients were aseptically collected, refrigerated, and tested within two weeks after collection.

Test methods: In general, the tests were performed as described by Parlett and Youmans[13] with some further modifications suggested by Parlett[13] as follows:

1. Instead of a 2:1 dilution of antigen, the following dilutions were employed: 3:1, 1:1, 1:3, and 1:20. The unconcentrated D29 antigen and concentrated Parlett-Youmans antigen were accorded similar treatment in this series of experiments. Results were recorded as positive when any evidence of banding was observed in the neutral agar layer.

2. Final readings were made at forty-eight hours rather than at one week, any changes occurring after seven, fourteen, and twenty-one days being recorded for future analysis.

To assure that the tests were being properly performed and results correctly interpreted, serum which had been examined in this laboratory and sets of gel double-diffusion tubes which had been recorded were submitted to Dr. Parlett, who corroborated the findings.

Of 103 serum specimens examined, 47 (or 45.6 per cent) were positive with both antigens, and 38 (or 36.5 per cent) were negative with both antigens. However, an additional 10 (or 9.7 per cent) were positive with only the D29 antigen, and 8 (or 7.6 per cent) were positive only with the Parlett-Youmans antigen.

* * *

The primary purpose of these preliminary comparative studies was to determine the possible

[1] Carnes, W. H., and Raffel, S.: Bull Johns Hopkins Hosp, 1949, *85*, 204.

[2] Choucroun, N.: Amer Rev Tuberc, 1947, *56*, 203.

[3] Middlebrook, G.: J Clin Invest, 1950, *34*, 1480.

[4] Rothbard, S., *et al.*: Proc Soc Exp Biol Med, 1950, *74*, 72.

[5] Wadsworth, A.: J Immun, 1931, *21*, 313.

[6] Thurston, J. R., *et al.*: Amer Rev Tuberc, 1956, *76*, 563.

[7] Coulthard, H. L.: J Path Bact, 1923, *26*, 350.

[8] Wadsworth, A.: J Immun, 1925, *10*, 241.

[9] Parlett, R. C., and Youmans, G. P.: Amer Rev Tuberc, 1956, *73*, 637.

[10] Weiss, D. W.: Amer Rev Resp Dis, 1959, *80*, 676.

[11] Froman, S., *et al.*: Amer J Public Health, 1954, *44*, 1326.

[12] Froman, S., and Bogen, E.: Bact Proc, 1955.

[13] Parlett, R. C., *et al.*: Amer Rev Tuberc, 1957, *77*, 462.

use of phage lysates as serologic antigens. No effort was made at this time to determine the specificity or sensitivity of the D29 antigen as compared with the Parlett-Youmans antigen. There is a high level of agreement (82 per cent) in the results obtained in comparison trials of unconcentrated D29 antigen with concentrated Parlett-Youmans antigen. The comparability of the D29 antigen with the Parlett-Youmans antigen has been confirmed by Parlett.[14] In addition, phage lysate antigen was prepared in a fraction of the time required for the culture filtrate antigen preparation as described by Parlett. More recent observations indicate that even more rapid detection of results and greater intensification of precipitate bands could be obtained by using pervaporation-concentrated D29 antigen.

As shown by Boyden and Sorkin[15] and Thurston and Steenken,[16] different antibodies in serum from tuberculous patients or animals may be detected by using antigens of various compositions. These observations may be further indicated by the finding that 17 per cent of the serum specimens examined gave positive results in gel double-diffusion tests with one or the other of the two antigens employed. Because of the encouraging results obtained in these preliminary experiments, it is intended to explore further the use of the D29 antigen and other phage lysate antigens in the gel double-diffusion test and in other serologic tests for tuberculosis.

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[14] PARLETT, R. C.: Personal communication.

[15] BOYDEN, S. U., AND SORKIN, E.: Symposium on Experimental Tuberculosis, Little, Brown and Co., 1955, pp. 144–159.

[16] THURSTON, J. R., AND STEENKEN, W., JR.: Amer Rev Resp Dis, 1960, *81*, 695.

[17] This study was made possible by a grant from the Riverside County and California Tuberculosis and Health Association.

THE SIGNIFICANCE OF THE RESPONSE OF MICE TO IMMUNIZATION WITH VIABLE UNCLASSIFIED MYCOBACTERIA[1]

The results of the pioneering tuberculin test studies of Palmer and Edwards and associates,[2–12] which have been confirmed recently by the results of tuberculin tests in college students by Sartwell and Dyke,[14] in medical and dental students by Youmans and associates,[15] and in medical students by Smith,[16] have provided convincing evidence that a high proportion of the people in many areas of the United States have been infected naturally with microorganisms of at least one general group (*Group III*)[17] of unclassified (so-called "atypical") mycobacteria. A very small proportion of the people so infected apparently will develop pulmonary disease resembling tuberculosis. The remainder may show a low degree of hypersensitivity to tuberculin prepared from cultures of *Mycobacterium tuberculosis* and a higher degree of hypersensitivity when tested with tuberculin prepared from one of the cultures of *Group II* unclassified mycobacteria. There is reason to believe that natural infection with *Group I* unclassified mycobacteria also may be widespread among human beings. The evidence for the occurrence of significant numbers of *Groups II* and *IV* unclassified mycobacterial infections in humans is less convincing.

This situation raises a question of great fundamental importance. Do those persons who naturally acquire an inapparent infection due to unclassified mycobacteria also develop an increased immunity to infection with virulent human tubercle bacilli? If the answer to this question should be in the affirmative, the implications, especially in regard to the epidemiology and control of tuberculosis, would be far reaching.

An essential preliminary step in the search for an answer to the above question is to measure experimentally in lower animals the immunogenicity of representative cultures of unclassified mycobacteria.

In this report, results will be given of preliminary studies which were designed to determine whether a measurable degree of acquired immunity to infection with virulent human tubercle bacilli could be produced in mice by injecting viable cells of several types of unclassified mycobacteria.

Male mice of the CF_1 strain, weighing between 18 and 22 gm., were injected intraperitoneally with different numbers of young viable cells of the strains of unclassified mycobacteria listed in table 1. Twenty-eight days later these animals were challenged intravenously with 1.0 mg. of a culture of the H37Rv strain of *M. tuberculosis*. The time in days of death of each mouse from tuberculosis was recorded and the immunizing efficacy determined by noting the proportion of mice of each group which survived thirty days. Complete details of this standardized immunity testing procedure may be found in another publication from this laboratory.[18]

At four and eight weeks after vaccination, 5 vaccinated, but unchallenged, mice of each group were sacrificed and autopsied to detect any gross pathologic evidence of infection which might have been produced by the viable unclassified mycobacteria used for vaccination.

Before sacrifice the weight of each mouse was recorded. A normal rate of weight gain was maintained by these vaccinated mice over the eight-week period. All of the mice were normal in appearance and activity at the time of sacrifice.

Autopsy revealed that all vaccinated mice had

[1] Aided in part by research grant E-1636 from the Institute of Allergy and Infectious Diseases of the National Institutes of Health, U. S. Public Health Service; and in part by a research grant from the Tuberculosis Institute of Chicago and Cook County.

[2] EDWARDS, L. B., *et al.*: Public Health Rep, 1958, *63*, 1569.

[3] GODDARD, J. C., *et al.*: Public Health Rep, 1949, *64*, 820.

[4] PALMER, C. E., AND STRANGE PETERSEN, O.: Public Health Rep, 1950, *65*, 1.

[5] PALMER, C. E., *et al.*: Public Health Rep, 1950, *65*, 1111.

[6] PALMER, C. E.: Amer Rev Tuberc, 1953, *68*, 678.

[7] WHO TUBERCULOSIS RESEARCH OFFICE: Bull WHO, 1955, *12*, 63.

[8] WHO TUBERCULOSIS RESEARCH OFFICE: Bull WHO, 1955, *12*, 85.

[9] EDWARDS, L. B., AND KROHN, E. F.: Amer J Hyg, 1957, *66*, 253.

[10] EDWARDS, L. B., AND PALMER, C. E.: Amer J Hyg, 1958, *68*, 213.

[11] EDWARDS, L. B., AND PALMER, C. E.: Amer Rev Resp Dis, 1959, *80*, 747.

[12] PALMER, C. E., *et al.*: Amer J Hyg, 1960, *71*, 218.

[13] EDWARDS, L. B., *et al.*: Acta Tuberc Scand, 1959 (Supplement 47, p. 77).

[14] SARTWELL, P. E., AND DYKE, L. M.: Amer J Hyg, 1960, *71*, 204.

[15] YOUMANS, G. P., *et al.*: Amer Rev Resp Dis, 1960, *82*, 114.

[16] SMITH, D. T.: Amer Rev Resp Dis, 1961, *83*, 213.

[17] RUNYON, E. H.: Med Clin N Amer, 1959, *43*, 273.

[18] YOUMANS, G. P., AND YOUMANS, A. S.: J Immun, 1957, *78*, 318.

TABLE 1

COMPARATIVE IMMUNIZING POTENCY OF UNCLASSIFIED MYCOBACTERIA FOR MICE OF THE CF_1 STRAIN

Mycobacterial Strain	Amount Injected	Number of Mice	Number of S-30 Mice*	Number of D-30 Mice†	Per Cent of S-30 Mice
	mg.				
Photochromogen‡ (*M. kansasii*) (*Group I*)	1.0	40	30	10	75.0
	0.1	38	28	10	73.6
	0.01	39	26	13	66.6
	0.001	19	5	14	26.3
Scotochromogen‡ (*Group II*)	1.0	19	5	14	26.0
	0.1	20	2	18	10.0
	0.01	20	0	20	0.0
Battey‡ (*Group III*)	1.0	38	14	24	36.8
	0.1	39	19	20	48.7
	0.01	39	15	24	38.4
Battey P-39§ (*Group III*)	1.0	20	10	10	50.0
	0.1	20	11	9	55.0
	0.01	19	2	17	10.5
M. avium§ (No. 2)	1.0	20	11	9	55.0
	0.1	20	12	8	60.0
	0.01	20	10	10	50.0
Rapid grower‡ (*Group IV*)	1.0	20	0	20	0.0
	0.1	19	0	19	0.0
	0.01	20	0	20	0.0
Controls	None	40	1	39	2.6

* S-30 = Survived 30 days

† D-30 = Died in less than 30 days

‡ Received from Dr. William Lester, Suburban Cook County Tuberculosis Sanitarium and Hospital, Hinsdale, Illinois.

§ Received from Dr. Ernest H. Runyon, Veterans Administration Hospital, Salt Lake City, Utah.

spleens about two to five times normal size. A few enlarged mesenteric lymph nodes (1–5 mm. in diameter) were noted in all vaccinated mice except those injected with the scotochromogen (*Group II*) and the rapid grower (*Group IV*). The enlarged mesenteric nodes were most numerous in the mice vaccinated with *M. avium*. No other gross evidence of infection was noted in any of the mice except in those vaccinated with *M. kansasii* (*Group I*). In these animals numerous small typically tuberculous pulmonary lesions were present in 3 of the 5 animals killed four weeks after vaccination. At eight weeks, all of the 5 mice sacrificed had pulmonary lesions which were appreciably larger and more numerous than those noted at four weeks. The pulmonary disease was minimal in nature, however, since these mice at the time of sacrifice had maintained their weight and showed no other evidence of illness.

All other data have been entered in the table. Two experiments were performed at different times with *Mycobacterium kansasii* and the Battey (Lester) strain but, since the results did not differ significantly, these data have been pooled.

Even small doses of *M. kansasii* and of the two Battey strains induced a significant amount of acquired immunity to tuberculous infection. The effectiveness of *M. kansasii* approached that obtained in similar tests using BCG vaccine.[18] This pronounced immunogenicity may have been related to the presence of pulmonary lesions in the mice vaccinated with this strain, a finding which suggests an appreciable degree of *in vivo* microbial multiplication and, therefore, perhaps a greater immunogenic stimulation. The immune response produced in the mice by the two Battey strains was of a lesser degree than that produced by *M. kansasii*, but was quantitatively similar to that which was obtained in this laboratory with the variant H37Ra strain,[19] and was equivalent to that produced by *Mycobacterium avium*. This last microorganism was included in these experiments because of the apparent close relationship of the avian strains to the Battey strains.[20–21]

On the other hand, the scotochromogen culture was only slightly immunogenic, and the rapid grower was devoid of any immunizing capacity. This last finding confirms results obtained previously with rapidly growing mycobacteria.[18]

* * *

These results demonstrate that unclassified mycobacteria of certain *Groups I* and *III* will stimulate the production in mice of a high degree of acquired immunity to subsequent infection with virulent human tubercle bacilli. These data do not, however, provide evidence for the immunogenicity of these microorganisms in human beings, but it does not appear unreasonable to accept, as a working hypothesis, that an appreciable degree of immunity would develop in persons naturally infected with these unclassified mycobacteria. This hypothesis also derives support from the fact that the only unclassified mycobacteria which produced a really significant degree of immunity in mice were those which have consistently been found to be

[18] RUNYON, E. H.: Tuberculosis Laboratory Methods, Veterans Administration, 1958, p. 14.

[19] BOGEN, E.: Quarterly Progress Report, Veterans-Administration-Armed Forces Study on the Chemotherapy of Tuberculosis, 1958, *53*, 69.

[20] BOJALIL, L. F., AND CERBÓN, J.: Amer Rev Resp Dis, 1960, *81*, 382.

concerned with inapparent infection and/or disease in man.

If, as a result of natural infection, both the photochromogenic *M. kansasii* and the Battey unclassified mycobacteria do produce a high degree of immunity in man to infection with *M. tuberculosis*, then it would follow that from 15 to 74 per cent of the people of the United States may be naturally immunized against tuberculosis by the time of early adulthood, since tuberculin testing surveys indicate that these proportions of Navy recruits, depending on the state from which they came, may be infected with the Battey microorganisms alone.[22]

It should be kept in mind that natural active immunization does not constitute a new concept. For many years it has been recognized that immunity, developed as a result of "inapparent" or "subclinical" infection, is probably responsible for much of the higher resistance of older groups of humans to such infectious diseases (among many others) as diphtheria, poliomyelitis, and endemic typhus; although in these instances the immunizing agent is believed to be the same infectious agent that causes the specific disease, rather than a different but closely related species. In this connection, inapparent infection with *M. tuberculosis* itself frequently has been used as an explanation for the increased resistance which has been noted in certain human population groups.

Direct evidence for the presence of a large nonspecifically immunized human population in the United States because of inapparent infection with unclassified mycobacteria is completely lacking. It is to be hoped that data will be accumulated in the future which will indicate whether the incidence of tuberculosis is appreciably less in people naturally infected with unclassified mycobacteria than in persons not so infected.

The very real possibility, however, that large numbers of naturally actively immunized people exist in the United States due to infection with unclassified mycobacteria raises the interesting question of what influence this situation may have had on the steady decline in incidence in tuberculosis which has been evident in this country ever since reasonably reliable morbidity rates have been available. The factors responsible for this persistant decline in incidence of this disease in the United States have never been clearly understood. In a recent review entitled "The Arcana of Tuberculosis," Grigg[22] again has pointed out that this steady decline apparently has been independent of the efforts mankind has spent on control of the disease. Grigg believes that the building up of a more resistant (immune) population over the years because of the elimination of susceptibles by the disease itself is the most important factor. The presence of a large, and possibly gradually increasing, nonspecifically immunized population because of infection with unclassified mycobacteria might furnish an equally good or perhaps even better explanation. Ironically, if found to be true, this also would provide vindication for the principle of control of tuberculosis through mass immunization with viable, attenuated mycobacteria, a principle which has been rejected by the majority of medical opinion in this country for many years.

In summary: Mice of the CF_1 strain were vaccinated with representative cultures of unclassified mycobacteria of *Groups I, II, III,* and *IV*. A high degree of immunity to infection with *Mycobacterium tuberculosis* developed only in those animals vaccinated with *Group I* (*M. kansasii*) and *Group III* (Battey) strains.

In view of these results and of the apparent widespread occurrence of inapparent infection in human beings with these unclassified mycobacteria, it has been suggested that a large human population may exist in the United States nonspecifically naturally immunized against infection with *M. tuberculosis*. The suggestion also has been made that this situation may have played a large role in the rapid decline in tuberculosis morbidity in this country.

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[22] GRIGG, E. R. N.: Amer Rev Tuberc, 1958, *78*, 151; 426; 583.

Correspondence

DETERIORATION OF ISONIAZID IN FROZEN SERUM SAMPLES: A CONFIRMATORY NOTE

To the Editor of the American Review of Respiratory Diseases:

The recent publication by Poole and Meyer on the deterioration of isoniazid in frozen plasma samples[1] prompts me to submit a confirmatory communication in order that this important observation will receive its proper recognition.

When performing isoniazid fall-off determinations, we attempted to facilitate laboratory scheduling by freezing serum samples for periods of one to seven days. Using the Poole and Meyer 1-*f*, 2,4-dinitro benzene method for the isoniazid fall-off procedure,[2] we soon observed that these frozen samples gave us values below the customary ones done on freshly drawn specimens. The data for 2 subjects whose sera were measured immediately and compared with a second portion, frozen for one week and then measured, are given in table 1. Determinations were done in duplicate with good agreement. Blanks were frozen when indicated.

A batch of pooled serum containing isoniazid was divided into several aliquots. Isoniazid determinations were done immediately, and twenty-four and seventy-two hours later, on frozen aliquots. The values are given in table 2. Both tables show a considerable decline in drug concentrations. We therefore abandoned the practice of freezing samples and discarded these data.

Isoniazid deterioration was found by Poole and Meyer to be accelerated in plasma when compared with aqueous and phosphate-buffered solutions. The isoniazid in the latter two media was stable below pH 7.0, but showed slight decomposition at pH 7.0, and significant decomposition at pH 8.0. Our blood samples have been found to reach a pH of 8.0–8.3 after standing several hours to allow clotting. Increased deterioration at high pH values was observed to occur in phosphate-buffered solutions by Winder and Denneny.[3] They have thoroughly studied the auto-oxidation of isoniazid in solution and have concluded that Mn^{2+} or Cu^{2+} catalyze these reactions, depending partly on the buffer used. The reaction gives an approximately first-order decay constant which decreases with time. This is compatible with the decay pattern noted by us and that observed by Poole and Meyer. Possibly here as well, cleavage to the acid catalyzed by trace metals in serum is responsible for this process. Reactions of isoniazid with other substances in serum must be quite common. We have recently observed spectrophotometrically that the drug reacts with thioglycolate in mildly alkaline media, in the presence of divalent cations, particularly Cu^{2+}.

The importance of these observations to the validity of bioassay or chemical determinations of isoniazid in serum samples subjected to any delay prior to their determination, even when maintained in a frozen state, is obvious and reflects serious doubt on data compiled from samples sent from referring hospitals to a reference laboratory. The bioassay technique[4] could give an erroneous impression of isoniazid stability either through failure of the method to detect changes of this magnitude or because of the appearance of other substances toxic to the tubercle bacillus as isoniazid deteriorates. A closer look here seems indicated.

The writer would also like to record one further observation. The effect of the Poole and Meyer method on hydrazones of serum isoniazid has not been described. A 10 γ per ml. serum sample of the pyruvic hydrazone of isoniazid[5] (referred to the hydrazide moiety) was measured by this method. In order to solubilize the hydrazone, it was necessary to dissolve it first in 0.1 M Na_2CO_3. One milliliter of this was then added to 49 ml. of pooled serum. The final pH was 9.7. With the Poole and Meyer method these conditions resulted in a figure for free isoniazid corresponding to 44 per cent of that contained in the hydrazone. It is probable that the five-minute period of heating at 80°C. causes this much hydrolysis.

This observation indicates that the Poole and Meyer method will measure some of the isoniazid hydrazones, as well as the unchanged drug. The other major products have already been eliminated from consideration. The proportion of isoniazid present as hydrazones in serum during the metabolism of the drug has not been measured, but it is probable that it is low, since they constitute only a small fraction of the

[1] Poole, F., and Meyer, A. E.: Proc Soc Exp Biol Med, 1960, *104*, 560.

[2] Jenne, J. W.: Amer Rev Resp Dis, 1960, *81*, 1.

[3] Winder, F. G., and Denneny, J. M.: Biochem J, 1959, *73*, 500.

[4] Bell, J. C., and Riemensnider, D. K.: Amer Rev Tuberc, 1957, *75*, 992.

[5] The hydrazone was tested at the suggestion of Dr. John H. Peters, Christ Hospital Institute of Medical Research, Cincinnati, Ohio, and provided by him. It had been found pure on assay.

TABLE 1

EFFECT OF FREEZING SERUM SAMPLES ONE WEEK AT −20° C. ON THE ISONIAZID CONCENTRATION

(Samples were obtained during the isoniazid fall-off procedure for half-life determination.)

Minutes following Injection of 5 Mg. per Ml. of Isoniazid Intravenously	γ Per Ml. of Isoniazid	
	Immediate	Seven Days Later
30	5.8	3.7
90	3.3	1.7
150	2.4	1.2
30	6.9	4.8
90	5.4	3.2
150	4.3	2.5

TABLE 2

EFFECT OF SHORT PERIODS OF FREEZING SERUM ON ISONIAZID CONCENTRATION IN POOLED SERUM

Immediate	9.4 γ per ml.
24 hours	5.8 γ per ml.
72 hours	4.8 γ per ml.

total metabolites in urine.[6] Furthermore, other methods currently used for serum isoniazid[7,8] involve dilute acid which probably disturbs the acid-labile hydrazones. It is quite possible that bioassay measurements reflect these substances as well, perhaps due to subsequent hydrolysis of this compound. The use of the Poole and Meyer method in the intravenous fall-off procedure should be considered sufficiently accurate for the classification of patient inactivation status. Its use in more precise metabolic studies, such as our studies of isoniazid renal clearance,[9] may lead to more significant errors.

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[6] HUGHES, H. B., *et al.*: Trans 14th Conf on Chemother Tuberc, VA-Army, and Navy, 1955, p. 217.

[7] MAHER, J. R., *et al.*: Amer Rev Tuberc, 1957, *76*, 852.

[8] PETERS, J. H.: Amer Rev Resp Dis, 1960, *81*, 585.

[9] JENNE, J. W., *et al.*: Trans 19th Conf on Chemother Tuberc, VA-Armed Forces, 1960, p. 34.

EFFECT OF POTASSIUM PARA-AMINOBENZOATE IN SARCOIDOSIS[1]

To the Editor of The American Review of Respiratory Diseases:

It is well known that spontaneous clinical improvement and complete clearing of roentgenographic signs is a common occurrence in sarcoidosis. In a minority, however, sarcoidosis is a progressive and serious disease. Corticosteroid drugs have been helpful in ameliorating symptoms of sarcoidosis and have prevented serious damage to eyes, heart, and kidneys. These drugs have not been shown to be effective in preventing or reversing established pulmonary fibrosis secondary to sarcoidosis.

The successful use of potassium para-aminobenzoate in scleroderma and other diseases characterized by fibrosis reported by Zarafonetis,[2] led to a trial of this agent in the treatment of the fibrosis due to sarcoidosis. Cohen, Lambert, and Molthan reported on the use of potassium para-aminobenzoate in fifteen patients with sarcoidosis.[3] They indicated that cough, dyspnea, and malaise decreased in fourteen patients, while partial or complete clearing of roentgenographic abnormalities occurred in thirteen patients.

Since spontaneous improvement is so frequent in subacute sarcoidosis, it appeared that a more rigorous test of the value of this agent would be a trial in patients with chronic sarcoidosis. Potassium para-aminobenzoate was administered to 22 patients who had had their disease for two or more years. Twenty-one patients had previously been treated with adrenal steroids; 9 required constant maintenance therapy. The dose of potassium para-aminobenzoate was 12 grams daily given in four divided doses in capsules or as a powder dissolved in cold water.[4]

Of 22 patients started on therapy, 10 received medication for less than three months, 3 received medication for 4 to 6 months, and 9 patients were

[1] The Sarcoid Clinic of Jefferson Medical College is supported by grants from the National Tuberculosis Association, the Delaware Anti-Tuberculosis Society, and the Pennsylvania Trudeau Society.

[2] ZARAFONETIS, C. J. D., Ann Intern Med, 1959, *50*, 346.

[3] COHEN, R. V., *et al.*: The use of "Potaba" in sarcoidosis, presented at annual meeting of the American Trudeau (now Thoracic) Society, Eastern Section, Boston, Massachusetts, 1958.

[4] Generous supplies of potassium para-aminobenzoate in the form of Potaba were made available by Mr. Michael Fuhrmann of Glenwood Laboratories, Inc., Bergenfield, New Jersey.

treated for a period of time exceeding 6 months. The average duration of treatment was 5.5 months.

Three patients showed subjective improvement, with decrease in cough and dyspnea. In 2 of these, no change was noted in roentgenographic findings while, in the third, progression of fibrosis occurred. Eight patients felt worse while taking the drug; in 2 of these patients, roentgenographic progression was noted while, in the other 6, the roentgenographic findings were unchanged. Eleven patients showed no change subjectively, nor were any changes noted on serial chest films.

Seven patients stopped the drug because of gastrointestinal symptoms. Despite attempts at camouflaging the rather unpleasant metallic taste, most of the patients in this group tolerated the medication for less than three months. In 9 patients the medication was discontinued because of progression of cough, dyspnea, and weight loss, or worsening of skin lesions. When prednisolone was substituted, symptomatic relief was obtained in a majority of patients. Three of the 22 patients died during the study from respiratory failure and chronic cor pulmonale.

Two patients experienced marked hemoptysis after seven and eight months of treatment, respectively. A third patient who received the drug for less than one month had a massive gastric hemorrhage requiring gastrectomy. Sections of the stomach showed sarcoid granulomas. It is probable that these complications were secondary to the extensive sarcoidosis rather than related to the use of potassium para-aminobenzoate.

In summary: Twenty-two patients with chronic pulmonary sarcoidosis were treated with potassium para-aminobenzoate for an average duration of 5.5 months. Three of the 22 patients experienced subjective improvement, while the remainder were unimproved or became worse. Chest roentgenograms were unchanged in 19 patients and showed progression in 3 patients. Gastrointestinal symptoms caused cessation of therapy in 7 patients, and progression of symptoms of sarcoidosis required a change of treatment in 9 patients. It is concluded that potassium para-aminobenzoate is ineffective in the treatment of chronic pulmonary sarcoidosis.

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Books

HERITABLE DISORDERS OF CONNECTIVE TISSUE. Second Edition. By Victor A. McKusick. Pp. 333, The C. V. Mosby Co., St. Louis, Missouri, 1960. Price, $12.00.

Since the publication of the first edition of this book in 1956, the application of genetics to medical problems has become increasingly fruitful in contributing to the understanding of many heretofore obscure diseases. Furthermore, interest in connective tissue, as in the "collagen diseases," has become more widespread as efforts are made to understand and explain the many disorders of which the pathogenesis is still unknown. Dr. McKusick's monograph contributes to both greater interest in and understanding of the conditions he describes as heritable disorders of connective tissue. Like the inborn errors of metabolism, their clinical manifestations are widespread and varied, and apparently inexplicable until the key is found; then they appear as logical consequences of a basic defect.

As in the first edition, the two introductory chapters provide for the clinician background information on the terminology and methods of modern medical genetics and on the biology of normal connective tissue. Subsequent chapters take up, in turn, the Marfan syndrome, the Ehlers-Danlos syndrome, osteogenesis imperfecta, pseudoxanthoma elasticum, and the Hurler syndrome. In each of these chapters a brief, interesting, historical survey precedes the presentation of the clinical manifestations, with individual case reports and pedigrees from both Dr. McKusick's personal experience and the literature; pathology, discussion of the probable basic defect and its mode of inheritance, and an extensive bibliography are also included. The concluding chapter discusses a variety of disorders which may prove to belong within this group of inherited conditions, and suggests lines for future investigation.

Although the organization of the material is unchanged from the first edition, there have been extensive additions throughout in both text and illustrations. Pertinent new material has been incorporated into each chapter, and the bibliographies have been brought up to date. The number of new references accumulated testifies to the active investigation going on in this field. New cases of each disorder have been added and illustrated, and the reports of many earlier cases have been extended.

As well as these general additions, normal standards are given for the upper segment–lower segment ratio, as measured in 2,104 white and Negro Baltimore school children, to aid in the evaluation of the body proportions for diagnosis of the Marfan syndrome. Earlier standards are inaccurate for contemporary whites and had not been determined for Negroes. The accumulation of larger numbers of carefully studied cases of the Hurler syndrome as well as recent experimental studies have made possible greater clarification of the probable basic defect. In the concluding chapter, seven case reports of fibrodysplasia ossificans progressiva have been included.

What makes this book remarkable is the integration of the careful accumulation of clinical detail and family pedigrees with pathologic findings and results of experimental work on connective tissue so as to reveal a pattern of the basic defect and its inheritance. Such a pattern increases understanding of these disorders and makes possible the detection of previously unrecognized, mildly affected individuals. Improved understanding also assists in the management and treatment of known cases and their prognoses, and provides the clinician with a basis for advising the families of patients as to the possibility of future occurrence. Of greater importance is the addition to the present knowledge of connective tissue in general.

The wide variety of clinical manifestations which occur in these conditions makes knowledge of these disorders valuable to physicians interested in all phases of medicine. By the excellence and clarity of his writing and by his superb illustrations, Dr. McKusick has added to the inherent interest of the material and has made his book a pleasure to read. He illustrates well the truth of his thesis that "clinical researches . . . demonstrate that the clinician can take his place with the so-called 'pure scientists' in the group which is trying to fit together the varishaped pieces of the intricate jigsaw puzzle that is connective tissue."

MARY BOON ROTHBARD
SIDNEY ROTHBARD
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THE HUMAN LUNG. Edited by Heinrich von Hayek and translated by Vernon E. Krahl. Pp. 372, Hafner Publishing Company, Inc., New York, 1960. Price, $13.50.

The Human Lung represents a novel approach to the study of the anatomy and physiology of the lung. Although the book was first written by von

Hayek in 1953, there have been a number of revisions since that time, including a small chapter on electron microscopy.

The reviewer finds it easy to agree with Krahl that this well-documented book, which contains many of the author's own meticulous studies, should be made available in English-speaking countries.

Each chapter in the book deals with a different component of the lung. Chapters on the thorax, trachea, and diaphragm are also included. Each of these subjects is dealt with from the point of view of physiology, gross anatomy, and histology. The text is basically one of anatomy and histology, since the author is an anatomist. The intertwining of physiology is based upon the basic knowledge of the anatomy of the lung. Von Hayek's work differs from the usual textbook of anatomy in his extensive use of references in the text as well as references to the different points of view expressed.

The chapter on electron microscopy is particularly enlightening. The presence of a continuous alveolar epithelium and the discovery of bronchiole-alveolar communications is demonstrated by this technique. The latter should prove to be of great significance to physiologists. The presence of a continuous alveolar epithelium is a point of great interest since this cannot be demonstrated by conventional techniques.

The text is amply illustrated by gross photographs, photomicrographs, and a number of drawings. The index is completely adequate. The table of contents is broken down into a large number of categories, which is of great assistance to the reader. Each chapter is subdivided by numerous titles. The bibliography, which is extensive, is arranged by chronologic appearance in the text and then alphabetically.

This generally well-edited book should find its way to the shelves of those interested in diseases of the lung.

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Das Röntgenschichtbild. By A. Gebauer, E. Muntean, E. Stutz, and H. Vieten. Pp. 454, Georg Thieme Verlag, Stuttgart (in U. S. and Canada, Intercontinental Medical Book Corp., New York), 1959. Price, $35.25.

This work will undoubtedly fill an important gap in the European literature on tomography. It has been compiled by four of the most outstanding German and Austrian roentgenography specialists, each of whom has contributed previously to the literature on problems of roentgenographic diagnosis and therapy. The book is primarily designed for the advanced specialist who wishes to acquire further knowledge of the diagnostic possibilities of tomography. In an introductory chapter by Professor Vieten, the theoretic foundations of the various procedures are discussed. It must be emphasized that this article is not written for the beginner but for the resident who is already familiar with more than the basic facts of this topic. The clinical material, which has been accumulated by Professors Muntean, Gebauer, and Stutz, is divided into chapters according to the regions of the body. The details for each position are given and illustrated with pictures of excellent quality (a feature for which the books of the Georg Theime press are generally known). The chapter on the thorax compares anatomic whole body sections with roentgenograms taken in the same plane. The problem of structures which are recognized on the tomograms, but which lie anatomically in a different plane, is discussed in detail. Well-selected case histories which help to focus the reader's attention on the essentials of the diagnostic problem before him are a great advantage of the clinical part of the book. Indications and contraindications of the tomogram are discussed for every single position, and an extensive, up-to-date bibliography is given.

The American reader who has within reach numerous excellent standard texts on tomography in English will still derive benefit from consulting the present work for special questions.

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New York Hospital—
Cornell Medical Center
New York, New York

Negroes and Medicine. By Dietrich C. Reitzes. Pp. 400, Harvard University Press, Cambridge, Massachusetts, 1958. Price, $7.00.

This monograph describes medical education for Negroes and patterns of medical care given to Negroes and rendered by Negro physicians in fourteen selected communities in the United States. The subject is obviously a timely one. The monograph is extremely well written. There are excellent statistical studies presented in tabular form which make for quick and ready comprehension.

Since World War II, the limitation of Negro medical students has not been the result of discriminatory admission policies in medical schools nor limitation of physical facilities of the two Negro medical schools to train an increasing number of students. The limitation of students is a

result of inadequate preparation for medical school, inadequate financial support during the medical school period, and inadequate compensation both emotionally and financially for the Negro who pursues this prolonged educational course.

The actual number of Negro students in medical schools with predominantly white student enrollments has increased from 93 students in 20 schools in 1947 to 236 students in 48 schools in 1956. In general, applicants to medical schools from colleges exclusively for Negroes and applicants to the two Negro medical schools have lower medical college admission test scores than do all applicants to medical schools. It is thought that these differences are due to cultural factors. Statistics show, however, that qualified Negroes desiring to go into medicine are not being accepted. The author suggests that this probably reflects poor counseling for the Negro applicants with regard to proper selection of schools. The author considers financial aspects of the problem and concludes that, although Negro medical students receive a greater proportion of assistance from their families than any other Negro student group, the general economic base of Negroes as a group is much lower than that of white persons. One would wish for better documentation of this aspect of the problem.

The opportunity for postgraduate medical education is great for the young Negro physician today. In the 10-year-period from 1947 to 1956, the number of predominantly white hospitals which had graduates from Howard and Meharry medical schools rose from 8 to 46, an increase of 500 per cent. The shortage of Negro physicians remains acute. Negroes comprised 10 per cent of the total population in 1950, but Negro physicians constituted only 2.2 per cent of all physicians despite the increase in the absolute number of Negro physicians in the decade 1940 to 1950.

The major portion of this book describes patterns of medical care given to Negroes and provided by Negro physicians. Fourteen communities in all sections of the country were studied. The factors regarding integration in medicine were:

(*1*) The presence of predominantly Negro hospitals; (*2*) the presence of influential groups actively interested in integration; (*3*) the existence of opportunities for Negro physicians to establish professional contacts with influential white physicians; (*4*) the strategy used by Negro physicians in obtaining hospital appointments; (*5*) the supply of well-qualified and well-trained Negro physicians; (*6*) the professional relationships between Negro physicians, with particular emphasis upon referrals; (*7*) the general patterns of race relations in each community.

This book will be of value to the medical educator, the hospital administrator, the administrative officers of all medical organizations, and to all physicians who (if the reviewer may be permitted to editorialize!) are anxious and desirous to help integrate the Negro into all aspects of our society.

JOHN COLLINS HARVEY
The Johns Hopkins Hospital
Baltimore, Maryland

BOOKS RECEIVED

NEOPLASTIC DISEASE AT VARIOUS SITES. Introductory Volume. A Clinical Prospect of the Cancer Problem. Edited by D. W. Smithers. Pp. 232, E. & S. Livingstone Ltd., Edinburgh and London (The Williams & Wilkins Co., Baltimore, Maryland, U.S. agents), 1960. Price, $8.50.

RADIOISOTOPE MEASUREMENT IN NUCLEAR MEDICINE. By Frank H. Low. Pp. 67, Picker X-ray Corporation, New York, 1960. Price, $2.50.

RESPIRATION: PHYSIOLOGIC PRINCIPLES AND THEIR CLINICAL APPLICATIONS. Edited by Peter C. Luchsinger and Kenneth M. Moser. Pp. 505, The C. V. Mosby Company, St. Louis, 1960. Price, $15.75.

REGULATION OF THE INORGANIC ION CONTENT OF CELLS: Ciba Foundation Study Group No. 5. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Pp. 100, Little, Brown and Company, Boston, 1960.

SILICOSIS AND OTHER PNEUMOCONIOSES IN SWEDEN. B. Axel Ahlmark, Torsten Bruce, and Åke Nyström. Pp. 399 plus Appendix, Svenska Bokförlaget, Norstedts, Stockholm, 1960.

SURGICAL DISEASES OF THE CHEST. Edited by Brian Blades. Pp. 580, The C. V. Mosby Company, St. Louis, 1961. Price, $22.00.

THE YEARBOOK OF DRUG THERAPY. 1960–1961 Series. Edited by Harry Beckman. Pp. 587, The Year Book Publishers, Inc., Chicago, 1961. Price, $8.50.

American Thoracic Society

Medical Section of the National Tuberculosis Association

Tuberculin Test for Surveys

A Statement of the Committee on Diagnostic Skin Testing

Tuberculin surveys of school populations and other comparable groups are laborious and expensive and should be planned to yield the maximal amount of useful information. The best standardized tuberculin is PPD, and the best method of administration is the intracutaneous or Mantoux method. A dose of 0.0001 mg (5 TU) should be administered, and the reaction recorded in millimeters after forty-eight to seventy-two hours. Quantitative data collected in this manner make possible accurate comparisons of the tuberculous infection rate in different grades, at various ages, and in different states. This also makes possible accurate measurements of the tuberculin rate in the same school year after year. The quantitative readings identify, by their larger reactions, those individuals and their contacts who are more likely to have or to develop active disease.

The Committee is well aware of the search for a simpler, more convenient method of administering the tuberculin test. In the opinion of the Committee, the first requirement is adequate scientific proof that the frequency of the reactions produced by other methods approximates that produced by the 5-TU Mantoux test. Moreover, the same persons should be identified as reactors or nonreactors by both tests. Such comparable data are not now available for any of the presently proposed tests. The information available indicates that multiple-puncture methods, such as the Heaf test, are essentially strong-dose tests, and there is no scientific evidence that adjustments can be made by dilution to give results comparable to the 5-TU Mantoux test. Until such evidence is presented, the Committee does not recommend the use of the patch or multiple-puncture tests for survey purposes.

Robert J. Atwell
Lydia Edwards
Rena W. Harris
Julius B. Novak
David T. Smith
Samuel C. Stein
Michael L. Furcolow, *Chairman*

NOTICES

The following dates and locations of the next three annual meetings of the National Tuberculosis Association and the American Thoracic Society have been selected and approved:

1962—Miami Beach, Florida—May 20–24
1963—Denver, Colorado—May 12–16
1964—New York, New York—May 24–28.

SUBSPECIALTY BOARD FOR PULMONARY DISEASE

Qualifications of Candidates for Certification

The Committee on Medical Education calls to the attention of the members of the American Thoracic Society the changes which have been made in requirements for certification in the subspecialty of pulmonary disease and in the approval of listing of hospitals for training in all of the subspecialties of internal medicine. They believe that a clear understanding of this information obtained from the American Board of Internal Medicine and the American Medical Association is important to all who may be concerned.

Prerequisite: The applicant must previously have been certified by the American Board of Internal Medicine. Three years must have elapsed following such certification before he is eligible to apply for admission to the Subspecialty Board examination.

Training: The candidate should have devoted at least one full year to postgraduate education in a tuberculosis hospital or a general hospital with facilities for the care of tuberculosis, and at least another year in the practice of internal medicine with pulmonary disease as a major element of such practice.

Before admission to the certifying examination in pulmonary diseases, a candidate must possess certain specialized knowledge and must have acquired particular skills which would entitle him to be consulted as an expert in this specialty. This includes a thorough and intimate knowledge of the normal and pathologic anatomy and physiology of the pulmonary system; skill in history taking and physical examination; proficiency in the interpretation of pulmonary function tests and the roentgenographic diagnosis of thoracic conditions; and familiarity with special procedures and techniques used in the study of pulmonary diseases.

Above all, the candidate must be able to assimilate the information obtained from all of these sources in a manner which will lead logically to correct diagnosis. He must also have a thorough knowledge of the pharmacologic and therapeutic application of drugs used in the treatment of pulmonary diseases, and an intelligent comprehension of the place for and indications for other forms of treatment, especially surgery.

References: The candidate should give as references the names of physicians who are familiar with his training and experience in the field of pulmonary disease to the degree that they are able to offer a valid appraisal of his qualifications as a specialist, based on the requirements listed above. The application must be approved by all members of the Subspecialty Board in Pulmonary Diseases.

ANNOUNCEMENT ON DISCONTINUING APPROVAL OF HOSPITALS FOR RESIDENCY IN INTERNAL MEDICINE SUBSPECIALTIES

(*Excerpted from the* Journal of the American Medical Association, *October 8, 1960*)

Page 817:

At the request of the American Board of Internal Medicine, the Council on Medical Education and Hospitals has agreed to discontinue approving residency training programs in the four subspecialties of internal medicine. The corresponding subspecialty boards are not discontinuing certification in these subspecialties, however, and the requirements for subspecialty certification will be found published in detail in the appropriate section of this issue. Note that candidates are not eligible for admission to examination in a subspecialty until three years after certification in internal medicine. New applications for

approval of residency training programs in the subspecialties will no longer be accepted. Currently approved residency programs in the subspecialties may affiliate with general medical residencies* provided they wish to have their training programs identified in that manner.

Page 818: Changes in Listing Approved Residency Programs:

Subspecialties of Internal Medicine—The lists of approved programs in the medical specialties of allergy, cardiovascular diseases, gastroenterology, and pulmonary diseases carry the captions that the programs listed are approved until June 30, 1961. They will not be listed in future directories. The Board considers that candidates for subspecialty training are sufficiently mature to select their own types and locations for such training and expects that candidates and institutions can plan the kinds of programs needed after study of the requirements for qualification of candidates published elsewhere in this issue. Further advice regarding subspecialty training must be secured by correspondence with the Secretary of the American Board of Internal Medicine.

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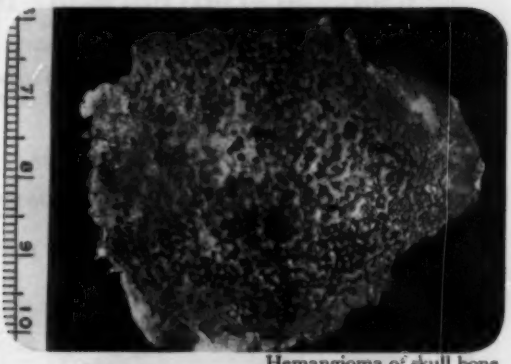
* By this is meant affiliation with a service in a hospital approved for internal medicine. The program for training in the subspecialty is to be accepted by the chief of service in Internal Medicine of that hospital. Suitable indication of approval for residency training in pulmonary diseases will be made in the *JAMA* listing of such hospitals under Internal Medicine.



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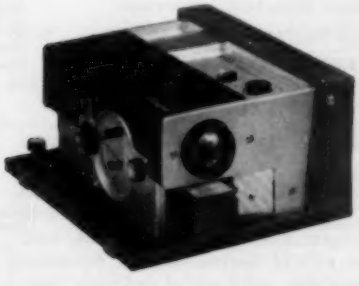

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United States	4,651
Canada	250
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PHYSICIANS

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	No.	%
No specialty	50	11.46
Starred pulmonary specialist	84	19.27
Pulmonary specialist	24	5.50
Starred internist	85	19.49
Starred surgeon	31	7.10
Surgeon	3	.68
Starred thoracic surgeon	27	6.19
Starred radiologist	7	1.60
Starred public-health specialist	5	1.14
Starred pathologist	3	.68
Starred pediatrician	3	.68
Starred otologist	2	.45
Starred and unstarred specialists in allergy, bacteriology, gastroenterology, hospital administration, industrial medicine, OALR, OBG, orthopedic surgery, physical medicine—one each	9	2.06
No information	17	3.89
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GP with interest in pulmonary diseases	24	5.50
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Full-time medical-school staff	7	1.60
Hospital administration	2	.45
Special research	1	.22
General public-health practice	10	2.29
Retired	3	.68
Not in practice	1	.22
No information	17	3.89
	350	80.19
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AGE OF PHYSICIAN

	No.	%
No information	17	3.89
No age given	1	.22
30–34	10	2.29
35–39	55	12.61
40–44	58	13.30
45–49	50	11.46
50–54	51	11.69
55–59	37	8.48
60–64	35	8.02
65 and over	36	8.25
	350	80.21
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POPULATION

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Under 10,000	56	12.84
10–25,000	39	8.94
25–50,000	44	10.10
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100–200,000	32	7.33
200–500,000	48	11.00
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BREAKDOWN OF INSTITUTIONAL SUBSCRIPTIONS

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3		2		5		2		0		1		0		Under 10,000	
3		2		1		0		0		1		0		10–25,000	
0		2		4		0		3		2		1		25–50,000	
1		1		1		0		1		2		2		50–100,000	
2		0		1		1		0		0		2		100–200,000	
3		6		0		0		0		1		4		200–500,000	
4		3		2		0		0		1		0		500–1,000,000	
2		4		1		1		2		0		6		Over 1,000,000	
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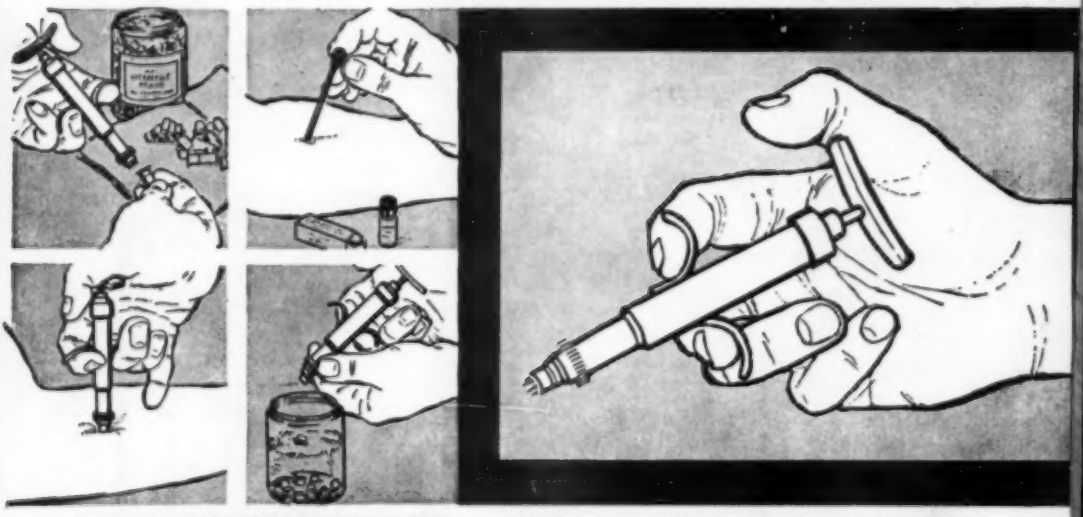

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